University of Alberta

Effects of plant species and herbivory on rhizosphere assemblages

by

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ABSTRACT

Plants can influence assemblages of soil-dwelling microbes and invertebrates through release of plant compounds from their roots. The amount and nature of exudates can be influenced by a range of biotic and abiotic factors. In a field test of the influence of plant species identity and seasonality on soil organisms, I found that only bacterial assemblages differed among species of herbaceous boreal plants at the Meanook Biological Station near Athabasca, Alberta. Seasonality had a large effect on invertebrates, with greater diversity and abundance later in the season. The effects of artificial herbivory on soil biota did not support bottom-up control as the invertebrate groups under the two herbivory treatments showed differing responses to the herbivory, highlighting the need for further research in this area.

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INTRODUCTION

Historically, ecologists have considered above-ground and below-ground organisms to be inhabitants of separate realms (Wardle 2002). The below-ground realm received relatively little attention, in part due to the difficulty of dealing with soil's opaque nature (Giller 1996). However, recent years have brought increased interest in soil and the interactions that occur therein, and a growing trend has developed to link these two domains in order to examine how they interact (Wardle 2002; Wardle *et al.* 2004). We now recognize that the above- and below-ground systems are inextricably linked, and that to understand what is happening in one, we must know what is happening in the other. This linkage is primarily through plants, which exist simultaneously above- and below-ground, and act as the principal connectors in this system (Wardle *et al.* 2004). Specifically, plants shunt sugars and other compounds from the leaves where they are produced, to the roots where they take part in root growth, and can be released into the soil as exudates that microbes then consume. As well, roots absorb water and nutrients that are necessary for whole plant growth, and so the plant can be affected, either positively or negatively, by the soil biota that regulate nutrient availability (Wardle *et al.* 2004).

The exudates that plants shunt below-ground are essential for soil organisms: these compounds enter the rhizosphere, the soil zone directly adjacent to the root, and serve as a food source for soil bacteria and to a lesser extent fungi, which in turn are consumed by soil fauna (Wardle 2002). Due to these exudates, bacteria congregate and multiply in the rhizosphere and their predators in turn are disproportionately found near plant roots (Grayston *et al.* 1996; Garrett *et al.* 2001; Bertin *et al.* 2003; Moore *et al.*

2003). Root exudates are made up of carbohydrates, amino acids, aliphatic acids, aromatic acids, fatty acids, sterols, enzymes, vitamins, and unidentified bacterial stimulators (Grayston *et al.* 1996). These exudates are also sometimes involved in antagonistic interactions, against bacteria that compete for nutrients (Grayston *et al.* 1996; Walker *et al.* 2003), against herbivores that attack the roots themselves (Topp *et al.* 1998), and against other plants in the vicinity through the process of allelopathy (Bertin *et al.* 2003)

Each plant species produces a specific blend of root exudates and with this, often harbours a unique assemblage of rhizosphere microbes (Wardle 2002). It has even been suggested that plants are able to adaptively manage the microbes present in their rhizosphere using these exudates (Walker *et al.* 2003). Legumes use their exudates to stimulate nodulation by *Rhizobium* bacteria, while other plants use anti-microbial compounds to defend themselves from soil pathogens (Walker *et al.* 2003). Since these exudates are important for other rhizosphere organisms, anything that causes a change in the abundance or nature of root exudates will likely affect the whole rhizosphere community. Some factors that can cause a change in the exudation pattern of an individual plant include plant age, site conditions, plant nutrition status, soil pH, water availability, temperature, light intensity, presence of particular microorganisms, and herbivory (Grayston *et al.* 1996). Indeed, herbivory by both above- and below-ground herbivores has been shown to increase the amount of exudates that are released into the rhizosphere immediately after the herbivory event (Holland *et al.* 1996; Grayston *et al.* 1996; Yeates *et al.* 1998; Grayston *et al.* 2001).

Soil biota, in particular the microbes and mesofauna (mites, nematodes, and springtails), play vital roles in the soil ecosystem through the breakdown of organic matter, mechanically moving and breaking up the soil, and through nutrient cycling (Coleman *et al.* 2004). Since 90% of above-ground net primary production enters the soil system, the decomposition that takes place is vital for returning nutrients to the plants, and thus drives the soil food webs (Coleman *et al.* 2004). The microbes (fungi and bacteria) are involved in decomposition as they are the ones that actively cycle the nutrients (Coleman *et al.* 2004). Detritivorous microarthropods, in particular springtails (Collembola) and mites (Acari), are also involved as they break the dead material into smaller chunks, thus increasing the surface area for the microbes to attack (Behan-Pelletier 1999). Mites and springtails are also able to transport microbes to new substrates in their gut, and deposit them in faeces. Microbivorous nematodes help to control the microbial population through consumption of bacteria (Coleman *et al.* 2004), and plant-parasitic nematodes can directly damage roots (Yeates *et al.* 1993).

In Alberta, few studies have examined the diversity, distribution, or ecology of soil microarthropods. Even our knowledge on what taxa exist in the province is limited (but see Behan-Pelletier and Eamer [2004] for oribatid mites). If we want to use these animals to monitor ecological change (ABMP 2005), we must first know what is present where and when. The overall objective of my study was to examine the effects that plants have on soil biota associated with plant rhizospheres. I studied soil organisms from the University of Alberta Meanook Biological Research Station (54° 37' N, 113° 21' W), north of Edmonton, both *in situ* and in a greenhouse study conducted at the University of Alberta. The effects of plant indentity on microbial and invertebrate

assemblages were investigated at the field site while the effects of simulated root and leaf herbivory was investigated in a controlled experiment. Since plant species have been shown to differ in their blends of exudates (Wardle 2002), one would predict that the bacteria and soil fauna would be selective in which plant species' rhizosphere they inhabit. For the herbivory experiment, since herbivory has been shown to increase the amount of carbon being exuded by plant roots (Holland *et al.* 1996; Grayston *et al.* 1996; Yeates *et al.* 1998; Grayston *et al.* 2001), this increase in nutrients should show bottom-up control in the soil system, i.e., microbes should increase due to increased nutrients, and the microbivores, followed by the carnivores, should respond by increasing their numbers as well.

STUDY OBJECTIVES

I had two main objectives:

1) To compare the bacterial and invertebrate assemblages in the rhizosphere of four boreal herbaceous plants in order to determine whether these assemblages are host-plant specific, and to assess how these assemblages vary over the growing season.
2) To test the hypothesis that above- and below-ground herbivory will increase the carbon pool in the soil, and that this increase will cause a sequential change in the abundance of soil organisms through a bottom-up control mechanism.

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EFFECTS OF PLANT SPECIES, SEASONALITY, AND LOCALITY ON BACTERIAL AND INVERTEBRATE ASSEMBLAGES FOUND IN THE RHIZOSPHERE OF BOREAL PLANTS

INTRODUCTION

A main theme in ecology is to search for patterns in the distribution and diversity of organisms, and to search for causes behind these patterns (Bardgett 2002). Many factors have been proposed to explain why organismal assemblages differ in different locations, including climatic variation (Whittaker 1975), latitude (review in Rohde 1999), habitat complexity (Kohn and Leviten 1976), and disturbance levels (Connell 1978). However, most investigations of patterns and determinants of diversity have focused on the above-ground ecosystem or the marine environment (Bardgett *et al.* 2005), and much less attention has been paid to variation in below-ground diversity. Plant species-richness has been suggested as an important driver of diversity in arboreal forest arthropods (Erwin 1982), and even on an individual plant, the arthropods present can vary due to the geographic range and competition between arthropods (Ødegaard 2000). Plants occupy two realms, both the above- and below-ground systems, and a question that has recently captured the attention of soil ecologists is whether above-ground plant diversity has an effect on below-ground organismal diversity (Wardle 2005).

The soil fauna includes transient individuals that spend only part of their life cycle below ground, either to lay eggs or as feeding larvae, as well as those that spend their entire life cycle permanently below ground (Wallwork 1970). The transient residents include coleopterans and dipterans, while permanent residents include the invertebrate groups Tardigrada, Nematoda, Collembola and Acari (Coleman *et al.* 2004). These invertebrates are classified as 'mesofauna', animals with body lengths between 0.16 –

10.4 mm (Wallwork 1970). Tardigrades and nematodes occupy the water film that exists around soil particles (Dindal 1990). Tardigrada, the water-bears, feed on microbes and other soil organisms, such as nematodes or even other water-bears (Dindal 1990). Nematodes can feed on microbes, be predators, or feed on the plant roots within the soil (Yeates *et al.* 1993). The Collembola, or springtails, are common soil dwellers and feed primarily on fungi and decaying plant matter, although some can be carnivorous (Hopkin 1997). The Acari, or mites, can be the most abundant soil microarthropod in many systems. The Oribatida, or beetle-mites, are the most common soil mites in forest systems (Maraun and Scheu 2000) while Prostigmata are generally the most common mite found in grasslands (Clapperton *et al.* 2002). Although microbes (bacteria and fungi) are the primary agents in nutrient cycling, the soil fauna also play a key role. They act as 'blenders', mechanically breaking up the litter into smaller units for the microbes (Coleman *et al.* 2004). Oribatid mites and collembolans deposit chitin-coated fecal pellets that have a high surface area to volume ratio, allowing the microbes a greater area to attack for nutrients (Coleman *et al.* 2004). Quality of litter also affects the rate of decomposition and nutrient cycling. Litter that is of high quality labile material with low amounts of lignin will cycle through the soil decomposer system at a faster rate than litter that is recalcitrant with higher lignin and phenolic content (Coleman *et al.* 2004).

Estimates of soil biodiversity are difficult to calculate as the species present, especially the smaller taxa and immature stages, are poorly known (Giller 1996). Current global estimates indicate that we have only described 4% of mites, 6% of nematodes, and 15% of Collembola (De Deyn and Van der Putten 2005). Albertan studies of the soil fauna have generally focused on agricultural soils, and identification of specimens has

been to a coarse level (Behan-Pelletier 2003). If there is an effect of the plant species identity on the assemblages of soil organisms, this would support attempts to use plant richness to estimate the diversity of the soil organisms, specifically the microbes and the mesofauna, the magnitude of which is currently unknown (Wall *et al.* 2005). To date, few studies have examined whether there are plant-specific assemblages of soil organisms, likely due to the difficulty of identifying the organisms to fine taxonomic categories. Of those that have, most have been in grassland systems (O'Lear and Blair 1999; Wardle *et al.* 1999; Korthals *et al.* 2001; Coulson *et al.* 2003; De Deyn *et al.* 2004), with a few in forest systems (birch forest: Huhta and Niemi 2003; oak forest: Lazarova *et al.* 2004) and the latter have focused on the effect of the trees as opposed to the understory vegetation. These studies have yielded mixed results, with some providing evidence for specificity (Wardle *et al.* 1999; Coulson *et al.* 2003; Lazarova *et al.* 2004; De Deyn *et al.* 2004), and others failing to (O'Lear and Blair 1999; Korthals *et al.* 2001; Huhta and Niemi 2003). A potential reason for this discrepancy in results could be due to the sampling method and collection intensity. Short-term studies get only a 'snapshot' of the system and may miss the peak diversity when plant-specificity might be most apparent. Another factor is how finely the fauna are identified. Most mesofauna are identified to a coarse level, to order or sub-order, which makes it difficult to observe changes in diversity that may be at the family, genus or species levels. This coarse level of identification is not ideal when looking at the functional diversity of these organisms, as the sub-order Oribatida contains mites with a variety of feeding types and life history strategies (Maraun and Scheu 2000; Coleman *et al.* 2004), therefore, conclusions on soil function based on this level of identification are not possible.

In this study, I test whether the functional assemblages of bacteria are plant-specific and whether the taxonomic diversity of the rhizosphere soil invertebrates are related to the species identity of a rarely studied group of plants, herbaceous perennials of the boreal forest-aspen parkland transition zone. In order to maximize the probability of detecting specificity, I sampled repeatedly over the growing season. Since it is believed that plant species have their own unique exudates (Wardle 2002), one might expect that the microbial assemblages around each plant species will also be unique. If the microbes are specific, it is also expected that the soil organisms, namely the nematodes, tardigrades, mites and collembolans, will also show specificity in which plant species' rhizosphere they inhabit.

METHODS

Study site

The study site was located at the University of Alberta's Meanook Biological Research Station (54° 37' N, 113° 21' W), near Athabasca, 130 km north of Edmonton, Alberta, Canada (Fig. 2-1). The research station is located in a National Wildlife Area in the transition zone of the boreal forest and aspen parkland eco-regions (MBRS 2004). The dominant tree species in the forest are trembling aspen (*Populus tremuloides* Michx.), balsam poplar (*Populus balsamifera* L.), and white birch (*Betula papyrifera* Marsh.). The understory is open with a variety of herbs and shrubs on the forest floor (Fig. 2-2).

On June 10th, 2004, two plots were established, approximately 200 m apart. Plot 1 was ~ 6 x 3 m and Plot 2 was ~ 5 x 5 m. Four species of herbaceous plants were chosen

based on availability at the sites: Canadian anemone (*Anemone canadensis* L.), fireweed (*Epilobium angustifolium* L.), northern bedstraw (*Galium boreale* L.), and wild vetch (*Vicia americana* Muhl.). I identified the plants using taxonomic keys for Alberta plants (Moss 1983; Johnson *et al.* 1995), however since vouchers were not taken, the identification of these plants should be treated with caution. In each plot, 8 individual plants of each species were identified and marked with flags. There were four sampling times: June 10th, July 14th, August 19th, and September 21st. At each time I took samples from two individuals per plant species per block (see *Sampling design* below). Thus there was a total of 64 samples at the end of the summer: 32 from each plot, 16 from each species, and 16 at each sampling date.

The amount of coarse woody debris (CWD) and the decay stages (Stelfox 1995) of each piece of CWD was measured at each site. The decay stages were based on a scale of 1 (newly fallen) to 7 (almost fully decomposed, no hard wood remaining).

Sampling design

At each individual plant immediately before soil cores were taken, soil moisture, light intensity, and soil temperature were measured. Soil moisture was taken using a soil moisture reader from Lincoln Irrigation Inc, which gave a reading on a scale of 0 (very dry) to 10 (very wet). To convert this into the amount of available water in the 10 cm depth of the soil core, the following equation was applied (Lincoln Irrigation chart, 2005):

Water (cm) = Maximum holding capacity at depth * (Lincoln meter reading * 0.1)

Where the maximum holding capacity is 2.667 cm (Lincoln Irrigation chart 2005) and the meter reading is between 0 and 10. Soil temperature was taken in centigrade using a digital waterproof thermometer. For both moisture and temperature, the instruments were placed in the soil directly adjacent to the plant stem. Light intensity was measured using an Extech Instruments Foot candle/Lux meter. The light sensor was placed at the top-most part of the plant to obtain the reading in foot candles. The foot candles were then converted to lm/m^2 using the equation:

$$lm/m2 = 9.290 * 10^{-2} \text{ foot candles}$$

Soil samples were taken using 2.5 x 10 cm steel corer (Fig. 2-3). At each plant, two cores were taken, one on the north and one on the south side directly next to the stem. These two cores were placed in a single cloth bag and labeled; this was then placed in a plastic bag to prevent cross contamination between samples. Sampled plants were marked so that they would not be re-visited. All the samples were left in a cool place overnight so processing could begin in the morning.

Invertebrate extraction

The two cores were first gently mixed in the cloth bag. Then, 1 g (wet-weight) of soil was removed for BIOLOG analysis (explained below), and 35 mL of soil was removed for nematode and tardigrade extraction using the Baermann funnel technique, and the remainder went into Berlese/Tullgren funnels to extract the arthropods.

Baermann funnel is a live extraction method that uses a funnel with a plastic tube clamped at the end (Dindal 1990). The sample is placed on a wire mesh in the funnel and water is added to cover the sample. The active nematodes and tardigrades then swim/crawl out of the soil and collect in the bottom of the plastic tubing (Dindal 1990). Modifications to the standard method were made: the sample was wrapped in cheesecloth and placed directly in the funnel, and the funnel was placed in a collecting jar instead of tubing. The Berlese/Tullgren method for extracting arthropods also uses the animals' movement in collection (Dindal 1990). The soil sample was placed on cheesecloth on a coarse mesh in a large funnel. At the bottom of the funnel was a collecting jar with 60% ethanol; a 40 watt light bulb above the funnel was used to desiccate the soil sample and cause the arthropods to travel downwards until they fall into the collecting jar.

The Baermann funnels were run for three days (Dindal 1990) with the jars stored in the fridge until sorted. I sorted the live nematodes and tardigrades with the aid of a dissecting microscope. Nematodes were fixed with hot FA 4:1 (formalin: alcohol, 4:1) and mounted either in glycerin or Bio Quip #6371 PVA (lactic acid, phenol and polyvinyl alcohol). The tardigrades were mounted in PVA. The Berlese/Tullgren funnels were run for 4 days, all arthropods were fixed in 60% ethanol, sorted and mounted in PVA for identification. Nematodes large enough to have identifiable mouthparts were assigned to trophic groupings (microbivore, carnivore, and plant parasitic); juveniles with unidentifiable mouthparts were categorized simply as 'larvae'. The tardigrades (Ramazzotti and Maucci 1983; Dastych 1988), collembolans (Christiansen and Bellinger 1981), oribatid mites (Balogh 1961; Balogh 1963; Balogh 1965; Balogh and Mahunka 1983; Subias and Balogh 1989) and the mesostigmatan mites (Walter unpublished key

2004) were identified to genera, while the remaining mites were identified to family (Walter and Proctor 2001) due to lack of identification keys beyond that level. The Prostigmata mite families and the Endeostigmata were verified by Dr. Heather Proctor (University of Alberta), the Oribatida were verified by Dr. Dave Walter (University of Alberta), Dr. Roy Norton (SUNY College of Environmental Science & Forestry) and Derrick Kanashiro (Alberta Research Council), the Mesostigmata were verified by Dr. Dave Walter, the Tardigrada were identified by Matthew Boeckner (Ph.D. candidate, University of Alberta), and the Collembola were verified by Dr. Jeff Battigelli (Earthworks Research Group). Assistance with placing the nematodes into trophic groups was provided by Matthew Boeckner.

BIOLOG procedure

BIOLOG EcoPlates are used to compare the functional diversity of the soil bacterial assemblages. The 96-well plate contains 31 different carbon sources replicated three times on the plate, with the three additional wells acting as controls with only water (Biolog 2000). A tetrazolium violet dye is also present in the wells so that when the carbon sources are utilized by the bacteria, the dye is reduced producing a purple colour in the well (Garland and Mills 1991). BIOLOG does not allow analysis of the soil fungal community as the fungi are unable to reduce the tetrazolium dye in the wells, therefore only obtain a measure of the bacterial community (Preston-Mafham *et al.* 2002). The soil to be analyzed is suspended in a sterile saline solution, diluted and added to each well of the plate. After a period of incubation, the plate is read and the colour development is recorded. The amount of colour development in each well gives an indication of the

respiration of the carbon source and so the metabolic potential of the community present in the soil (Garland and Mills 1991). Although each carbon source appears three times on the plate, they are not true replicates since they came from the same soil sample and are not independent of each other; therefore the average of the three wells is calculated. The area under the curve is then used for the analysis to account for differences in the lag time to colour development for each carbon source (Preston-Mafham *et al.* 2002). Multivariate statistics can then be used on the data to see if communities are similar or dissimilar. Changes in the pattern of carbon utilization would indicate differences in assemblage composition (Campbell *et al.* 1997).

The procedure I used was based on Yan *et al.* (2000), with a few modifications. One gram of soil was placed in 100 mL of sterile 0.85% NaCl and placed on a shaker for 3 hours. Five mL of the soil solution was diluted with 45 mL of sterile 0.85% NaCl to create a 1:10 dilution. I used a micro-pipette to add 120 μL of this solution to each of the 96 wells on an EcoPlate. No attempt was made to remove debris from the solution before inoculating the plates, since it is recommended to leave this suspension to ensure the entire bacterial assemblage is selected (Preston-Mafham *et al.* 2002). Once inoculated, the plates were placed in an incubator at 23°C in the dark. Plates were read at 48, 72, 96, and 120 hours after inoculation using a BIOLOG plate reader. The well colour was recorded at each time period so that the rate of colour development (= use of substrate by bacteria) for each well could be characterized, which is considered a better measure of the functional assemblage of bacteria than a single reading (Preston-Mafham *et al.* 2002). The average of the three replicates was calculated to avoid pseudoreplication. Negative numbers indicate less colour than the control water wells, and so were converted to zero

before any analysis took place. The carbon utilization was determined by calculating the area under the curve for each well over the 120 hours of incubation. This number is what was used for statistical analysis.

Statistical analysis

Abiotic site conditions – The soil moisture, light intensity and soil temperature were each analyzed separately using ANOVA in SPSS 11.5 (SPSS 2002). The fixed factors were sampling date, plot, and plant species. Tukey's post-hoc test was performed on significant factors to discover the differences between the levels of that factor.

BIOLOG – The area under the curve data was transformed into ranks ranging from 1 to 64 for each of the 31 carbon sources (the water well is omitted from analysis). In other words, for the sample in which there was least use (lightest colour) of a substrate the rank of 1 was given, whereas the sample in which there was most use (darkest colour) of that substrate received the rank of 64. The transformed data was then entered into PATN 3.03 (Belbin 1989) for analysis via ssh-MDS ordination (semi-strong hybrid multidimensional scaling). The distance matrix of samples was based on the Gower metric, which is useful for a continuous data set with few zeros. The ordination with the lowest stress out of 1000 random starts was selected for plotting. Analysis of Similarity (ANOSIM) was employed to test the differences between the two field plots, between the four plant species and the four sampling times in terms of carbon usage.

Invertebrates – The analysis of invertebrate assemblages was also done using PATN 3.03. Three data sets were examined: all invertebrates, nematodes only, and non-nematode taxa only (mites, springtails, and tardigrades). Nematodes were analyzed

separately because they were placed in feeding/developmental groups and not identified to taxonomic groups. Raw abundances were used in each case and the abiotic site conditions were added as extrinsic variables to see if they correlated with the invertebrate data. Taxa with only a single observation were removed before analysis. When the nematodes were analyzed separately, Gower metric was used; for all other analyses, Bray-Curtis was used (this metric is useful for data-sets with many zeroes). All analyses had 1000 random starts. ANOSIM was also performed to test if there were differences between plots, between plant species and between sampling times. Monte-Carlo Attributes in Ordination (MCAO), which assigns each variable a vector based on correlation, was performed to understand which invertebrate groups were important in creating the ordination or which abiotic site conditions were correlated with the ordination. For the ordination graphs, the final number of dimensions was chosen to reduce the amount of stress in the ordination. Two-dimensions would have been ideal, however, they resulted in stress values that were above the 0.2 threshold so three-dimensions were used in order to reduce the stress. Within the ordination graphs, the axes are standardized from randomly selected coordinates in the dimension although the relationship between points in coordinate space is not arbitrary (PATN 2005). However, a recent look into the axis scaling reveals that all the axes in 3-D are not equal and so some of the ordinations may be 'squished' and relationships slightly harder to view (PATN 2005). This problem has no bearing on the ANOSIM results. Univariate ANOVA with SPSS 11.5 (SPSS 2002) was performed on the abundance of all invertebrates in major taxa (to order for Collembola, Mesostigmata, Eutardigrada, and sub-order for Oribatida, Endeostigmata and Prostigmata), log-transformed to obtain a

normal distribution. Taxon richness was also analyzed, excluding nematodes since they were identified only to functional group, to see if there was an effect of plant species, season, or plot on the number of taxa found.

RESULTS

Site conditions

The main understory vegetation that categorized Plot 1, in addition to the study plants, were a variety of grasses, mosses, *Cornus canadensis* L., *Vaccinium* sp., *Fragaria* sp., *Pyrola* sp., *Rosa* sp., *Salix* sp., and *Cornus stolonifera* Michx.. The understory vegetation that categorized Plot 2, in addition to the study plants, included a variety of grasses, mushrooms, *Cornus canadensis* L., *Trifolium* sp., *Fragaria* sp., *Pyrola* sp., and *Rosa* sp..

ANOVA of the site conditions (light intensity, soil temperature, and soil moisture) indicated a significant effect of sampling time. Soil temperature was significantly different at all sampling times ($P<0.001$), with July having the highest and September having the lowest soil temperature (Fig. 2-4). For soil moisture and light intensity, while there were significant differences based on sampling time and plot (Figs. 2-5 and 2-6); however, the fact that they were instantaneous sampling measures, and not continuous over time, raises doubt about their biological significance on the soil organisms since these factors can vary greatly over a day and a season. For all the site conditions, there was no significant difference based on which plant species was sampled ($P>0.05$). All data for site conditions can be found in Appendix 1.

Bacterial assemblages

PATN analysis of BIOLOG data showed a significant difference in bacterial activity between the two plots (ANOSIM P=0.005; Fig. 2-7). The mean ranks per sample were calculated and compared between the two plots. Plot 1 had lower mean ranks than Plot 2 (Table 2-1). There was more CWD in Plot 2 (Table 2-2), which may explain the differences in bacterial activity. There was also a difference in light intensity between the two plots (Fig. 2-6), with greater light intensity in Plot 2, the plot with higher bacterial activity. Since the difference between the plots might have overshadowed the effects of other factors, the plots were separated and ranks reassigned for analysis.

When PATN analysis was done on Plot 1 alone, I was able to distinguish an effect of plant species, as well as an effect of sampling time (Table 2-3). For plant species, *Anemone* and *Galium* showed significantly lower bacterial activity than did *Vicia* (Table 2-3). For sampling time, June and July were similar, with August and September increasingly different (Table 2-3). When mean ranks are examined by plant species, we can clearly see the differences among them (Table 2-4). *Galium* shows the lowest activity with regard to carbon source usage, followed by *Anemone*, then *Epilobium*, and finally *Vicia*.

For Plot 2 plant species, the groupings that were found in Plot 1 were repeated in Plot 2 (Table 2-3). There was, however, no difference between the sampling times in regards to carbon usage. When the mean ranks were examined by plant species, the findings from Plot 2 were once again similar to those from Plot 1 (Table 2-4). Interestingly, the plant species with the higher bacterial activities, *Epilobium* and *Vicia*,

had very similar mean ranks in both plots. The other two species, *Anemone* and *Galium*, were more alike in Plot 2 then they were in Plot 1.

Invertebrate assemblages

Over the sampling season, a total of 2033 nematodes, 2209 mites, 185 springtails and 117 tardigrades were extracted from the 64 samples collected from Meanook. Further information and finer taxonomic identification can be found in Appendix 2. Voucher specimens of the arthropods will be deposited in the E.H. Strickland Entomology Museum at the University of Alberta, Edmonton, Alberta, Canada. Tardigrade exemplars will be deposited in the Freshwater Museum, Department of Biological Sciences, University of Alberta, Edmonton, Alberta. The nematodes had approximately equal numbers in each trophic group/developmental category: 504 microbivores, 499 carnivores, 498 plant parasitic, and 532 larval. The Oribatida was the most abundant mite group collected with 1506 individuals comprising 24 genera. The Mesostigmata were next with 314 individuals in 12 genera. The Prostigmata had 196 individuals in 13 families, the Endeostigmata had 101 individuals in 2 genera, and the Astigmata had 92 individuals in 2 groups. The 185 Collembola were divided among 10 genera, and the 117 Tardigrada were divided among 4 genera. The mean number of mites per m^2 in the Meanook forest works out to 70, 408/ m^2. During the sampling time with the highest abundance, September, this number increased to 116, 531/ m^2.

When all the organisms were analyzed together using PATN, there was no significant effect of plant species on the invertebrate assemblages ($P>0.05$). Unlike the bacterial assemblage, there was no difference between the two plots. There was,

however, a strong seasonal effect and soil temperature and light intensity were identified as significant vectors by MCAO (Table 2-5; Fig. 2-8). Figure 2-9 indicates which organism groups were significant in creating the ordination at $P<0.01$. Since there was such a strong effect of sampling date, the assemblages were reanalyzed for each sampling time to see if any other effects were being masked. These analyses also resulted in no significant effect of plant species on invertebrate assemblages (ANOSIM $P>0.05$; Fig. 2-10).

Nematodes were analyzed on their own to see if they showed any pattern over the sampling season. They were analyzed separately as they were identified to trophic and developmental groups as opposed to taxonomic groups. Sampling time was significant for all comparisons except for August and September, which were similar (Table 2-6; Fig. 2-11). Soil temperature was identified as a significant vector using MCAO and therefore is correlated with the ordination. The nematodes showed no specificity to plant species, with all ANOSIM pair-wise comparisons showing $P>0.5$ (Fig. 2-12). The nematodes were also similar in the two plots, with no significant difference between the two ($P=0.5275$)

The non-nematode taxa were also analyzed separately. Sampling time was significant for the taxa with all sampling times differing from each other (Table 2-7); soil temperature and light intensity were significant vectors in the ordinations, identified by MCAO (Fig 2-13). Figure 2-14 indicates which non-nematode taxa were significant at $P<0.01$ in creating the ordination. Plant species was not a significant factor, $P>0.1$ (Fig. 2-15). There was, however, a plot effect, with the two plots having significantly different

assemblages (P=0.0260) although when each plot was analyzed separately, there was still no effect of plant species (P>0.1).

The invertebrates were also analyzed using univariate ANOVA based on abundance at a coarser taxonomic level for each sampling time (Table 2-8). When the abundance of the various groups was analyzed, we see that for the mites, there was no difference in abundance between June and July, and that the Mesostigmata are the only group that had a significantly higher abundance in August when compared to the previous two months. The other groups are starting to diverge, but not until September do we see any that are significant greater than the first sampling times (see Table 2-8 and Figs. 2-16 & 2-17). The only exception to this is the Prostigmata, which show no significant differences in abundance over the season. The Collembola showed a similar pattern to the majority of the mites, with differentiation starting in August, and September being significantly greater compared to the beginning of the season (Fig. 2-18). For tardigrades, on the other hand, abundance in June was significantly less than in the other months, which were similar (Fig. 2-18). The nematodes were similar to the tardigrades with the June sampling time being significantly lower than the rest (Table 2-8). With the exception of the plant parasites, the nematode groups peak in abundance in July, and then decline slightly for the rest of the season (Fig. 2-19).

The taxon richness shows a similar trend to the rest of the results, with September being quite different from the rest of the sampling dates (Table 2-9). The nematodes were not included as they were identified only to functional groups. The Astigmata were excluded as well since they were represented by only two life stages. For the Oribatida and the Mesostigmata, there was no change until August and no significant difference

from the beginning of the season until September (Figs. 2-20 & 2-21). For the soft bodied mites (Prostigmata & Endeostigmata), we see the change starting in July, but still no significant difference until September (Fig. 2-21). The Collembola showed a decrease in July, and the tardigrades had the same pattern as for the abundance with June having low richness and the rest of the season with higher and similar results (Fig. 2-21). When all the taxa are combined, we see a continuous increase over the summer with twice as many taxa present in September as were present in June (Fig. 2-20).

DISCUSSION

Based on these results, variation in bacterial assemblages (as judged by patterns in carbon usage) partially supports the original hypothesis: there does seem to be an effect of plant species on the bacteria associated with the rhizosphere. The invertebrates, on the other hand, do not support the hypothesis that above-ground plant diversity would affect the below-ground faunal assemblages; however, seasonality did have a strong effect on invertebrate assemblages, with greater diversity and abundance later in the season. For the bacterial assemblages, seasonality was only significant in one plot, with seasonality not having a significant effect on the other plot.

Bacterial function

The difference seen between the two field plots in terms of bacterial activity could possibly be ascribed to different amounts of deadfall in the plots. Plot 2 showed higher bacterial activity and had a higher amount of coarse woody debris (CWD) associated with it than did Plot 1 (Table 2-1; Table 2-2). The deadfall could increase carbon inputs into

the soil through leaching, or branches may have been embedded into the soil (Spears *et al.* 2003; Ganjegunte *et al.* 2004; Homann *et al.* 2004). With a greater amount of carbon in the soil, it is possible this would cause an increase in the microbial activity and abundance. A recent study on the effects of forest debris showed a decrease in microbial activity when the forest debris was removed (Ruan *et al.* 2005), therefore highlighting the role of deadfall and litter inputs as nutrients for the soil microbes.

Once the influence of plot is removed we start to see the effect of the host plant. In both plots, the bacterial activity as measured by ranks was consistent for each plant species (Table 2-4). While we do not know the identity of the bacterial taxa for each of the four plant species, we can see that they show similar activity in terms of carbon source utilization under each of the target plant species between sites.

The effect of sampling date, unlike plant species, differed between the two plots (Table 2-3). Where Plot 1 showed an effect of date on bacterial activity, Plot 2 did not show any difference in activity levels over the summer. While the cause of this seasonal difference in unclear, it is possible that it is related to the amount of CWD in the plots. Whether slow and constant leaching of carbon from the CWD would cause this constant activity level in the bacteria is unclear, and warrants further study.

Some of my findings were consistent with previous research. *Vicia*, a nitrogen fixer, had the highest amount of bacterial activity within its rhizosphere as judged by the ranks of carbon use. Other researchers have also found greater abundances of bacteria, and bacterial feeders, under leguminous plants (Griffiths 1990; Viketoft *et al.* 2005). Since there is more nitrogen available under these nitrogen fixers, there is usually a

greater rate of bacterial growth than under non-leguminous plants (Grayston *et al.* 1998; Viketoft *et al.* 2005).

Although BIOLOG data does not tell us information on the abundance of the bacteria, higher rates of carbon utilization (i.e. larger area under the curve) could indicate higher bacterial abundance (Preston-Mafham *et al.* 2002). The activity levels are different between plant species, but whether this translates into differences in the microbial community is unknown. Other researchers have shown soil bacterial communities to be distinct under different plant species (Grayston *et al.* 1998; Wardle 2002), and so the differences in activity could be due to differences in bacterial community. The relative activity of the plant species in relation to each other was similar in the two plots, even though there was a significant difference in the activity levels between the plots. This could suggest that each plant species is supporting a different array of bacteria, although further tests using methods that determine taxonomic identity are needed. While this would have been ideal for this study, the cost of determining the taxonomic diversity of the soil bacteria was cost prohibitive.

While it was not addressed in this study, looking at differences in exudates of the plant species and understanding which exudates attract which bacteria would further our understanding of how this system works. Since I observed differences in the carbon utilization based on the BIOLOG analysis, it is reasonable to hypothesize that this may have been caused by differences in carbon sources released by the plant species. Further studies to identify the bacteria present under each plant would clarify whether the differences observed were due to differing bacterial abundances between plant species or if they were due to differences in bacterial identity between plant species.

Invertebrate assemblages

The invertebrates *en mass* show a definite seasonal pattern to their assemblage structure (time $P<0.001$), with no differences attributable to either plot or plant species (plot $P>0.05$; plant species $P>0.05$). For the nematodes and tardigrades, seasonal differences are due to the relative paucity of organisms in the June sampling, with higher numbers being extracted for the remainder of the summer (Figs 2-18 & 2-19). The reason for the low numbers of nematodes and tardigrades in the June sampling is unclear, although high mortality over winter is a possibility. Indeed, in a study looking at the effects of frost and freezing on soil communities, Sulkava and Huhta (2003) found the numbers of microbes, nematodes and microarthropods were lower in the spring after the soil temperature had dropped below 5-10°C. Since the observed soil temperature in my study had soil temperatures of ~10°C in June and ~8°C in September (Fig. 2-4), it is reasonable to assume that the temperature dropped even lower over the winter and therefore would have strongly negative effects on some soil animals. Another possibility for the low numbers in June is the over-wintering stages of the organisms. If the organisms over-wintered as eggs, then they may not have had time to hatch and become active before the first sampling time. Indeed, knowing the life history strategies of these organisms is important in understanding how diversity and abundance vary over the season.

Taxon richness and abundance of non-nematode taxa also increased over the summer season (Table 2-9; Fig 2-20). This result agrees with another study of Alberta soils by Clapperton *et al.* (2002), which showed a greater abundance of mites in October

than in June, and a greater number of mite families being represented in October. The taxon richness of all invertebrates in my study doubled from June to September; therefore, for those interested in 'snapshot' style surveys of organismal diversity in Alberta (e.g., the Alberta Biodiversity Monitoring Program, [ABMP 2005]) it would seem that in order to obtain the highest richness and abundance of soil invertebrates, samples should be taken later in the summer. This recommendation should be heeded cautiously until the pattern is shown to be repeatable across years and in other locations.

My results showed no variation in invertebrate assemblages based on plant species. Unlike my research, other studies have found a link between soil invertebrates and particular plant species, both for mites (Coulson *et al.* 2003; Badejo and Tian 1999; Badejo *et al.* 2002), and for nematodes (De Deyn *et al.* 2004; Viketoft *et al.* 2005). Although Coulson *et al.* (2003) found essentially the same mite and springtail species under the examined plants, it was the ranking of abundance of the arthropod species that showed predictable plant-specific patterns. Differences in densities of target plants may explain why my results do not match those of other studies. Badejo *et.al.* (2002) found a unique composition of mites under each of the three legumes in their study. I did not observe a unique assemblage under the legume, *Vicia*, however the study by Badejo *et al.* (2002) conducted under monocultures, which could explain their findings in contrast to my own as the understory in the Meanook forest did not have monocultures of the herbaceous plants. Badejo and Tian (1999) found differences in soil mites under four tree species. However, the tree species were in different stands and so there may have been other variables that caused the differences, not simply the tree species itself. De Deyn *et al.* (2004) found differences in nematode trophic groups under different plant species

when plant communities consisted of 1, 2, 4, 8, or 16 plant species. For the nematodes, plant identity was more important in structuring the community than plant species diversity; therefore it is likely resource quality, through plant exudates or litter, affected the nematodes. Viketoft *et al.* (2005) found both abundance and identity of nematodes to be affected by plant species. Their study was conducted on monocultures in the field, based on functional group: grasses, legumes, and forbs. Both nematode studies found the nematodes closest trophically to the plant (the plant feeders and microbivores) were most affected by plant species identity; the omnivores and carnivores were not affected by the plant species. My study did not reveal a difference in the trophic distribution of nematodes based on plant species. Perhaps the fact that the bacterivores and fungivores were not separated could have caused this, especially since the fungal mycelium could stretch further than plant roots and may have been ubiquitous in the area.

In a study looking at the effects of plant diversity on soil mites, St. John et al. (in press) found that a simple increase from one grass species to two correlated with an increase in the diversity of mites. They ascribed this to the increase in habitat diversity created by the competing plant roots, as habitat diversity is known to be positively related to mite species richness (Hansen and Coleman 1998). This allowed a greater variety and size of mite to be present under the two grass species as the competing root systems cause an increase in the soil habitat heterogeneity. This may have occurred in my study, and the intertwined roots may explain why there is no obvious specificity of the invertebrates: the invertebrates may be choosing the structure in the soil as opposed to specific plant rhizospheres.

In my study it did not appear that the invertebrates were choosing specific plant rhizospheres. In an experiment testing the effects of plant species removal on soil biota, Wardle *et al.* (1999) found that soil mesofauna were unresponsive to plant variables, that as long as plants *per se* were present, other factors (net primary production, whether plant species were removed, plant biomass, total carbon, total nitrogen, pH) did not affect these organisms. The mesofauna at Meanook may react in the same way; as long as there are plants to provide basic resources, the animals are content to exist near any species. It is also possible that the taxonomic level to which organisms were identified was too coarse to recognize species-specific responses of invertebrates to plant species. A final caveat is that with intertwined roots in the soil, it is difficult to sample only a specific plant species rhizosphere, an inherent problem with field studies in natural (non-monoculture) situations (De Deyn *et al.* 2004). In my greenhouse study (Chapter 3), I found that the two plant species used, fireweed and evening primrose (*Oenothera biennis* L.) each developed unique assemblages of invertebrates when grown separately in pots, so it is possible that plant species can affect assemblages of soil invertebrates, but this is not readily detectable in field conditions.

Seasonality

The tardigrades and arthropods in this study were affected by the sampling date with the general trend being an increase in abundance and diversity over the summer. Although nematodes also increased in abundance, we cannot make the diversity assumption as they were not identified to taxonomic groups. For bacterial assemblages,

Plot 1 showed differences in the sole carbon use with greater usage in August and September; Plot 2, however, did not show any seasonal differences.

Other studies on bacterial dynamics have also shown seasonal patterns in bacterial communities. Papatheodorou *et al.* (2004) found a strong seasonal pattern to the bacteria in northern Greece using BIOLOG plates. They found bacteria to be less active in the winter months, from November-December, compared to May. In a study in north-west Scotland, Grayston *et al.* (2001) had higher activity and abundance in November compared to earlier in the year. These results are similar to my own in that activity in Plot 1 was significantly higher in September than in June. However, it is still unclear why Plot 2 did not show a seasonality effect similar to Plot 1. As stated previously, this may be due to the amount of CWD between the plots, but this relationship has not been tested.

In a study on bacteria-eating nematodes in northern Greece, Papatheodorou *et al.* (2004) found the greatest abundance of the nematodes in September and October. This is similar to my results, which had the number of microbivores highest in July, followed closely by September. A study of population dynamics of Mediterranean soil arthropods, in particular an oribatid mite, found the highest abundance of the organisms occurred in late summer-early autumn (Stamou *et al.* 2004). This is also similar to my results which had the highest abundance in the September sampling period. In a study on the effects of temperature on soil fauna in a Finnish forest, Sulkava and Huhta (2003) also found the lowest number of soil organisms in the spring with abundances increasing over the summer to a fall maximum.

Based on my study and others, it appears that soil organisms are significantly influenced by seasonal variation. Most likely, this is due to temperature variation (Sulkava and Huhta 2002; Papatheodorou *et al.* 2004) exerting influences on the organisms and affecting their growth and nutrient sources. This is especially likely in Alberta where there is such a variation in temperature from summer to winter, there may be high numbers of invertebrates killed in the winter and so each spring the populations are reduced and it takes the summer to build up the numbers again. Another factor is the plant size and growth phase: earlier in the season, the plants are smaller, and do not have as much carbon to allocate below ground, but as the plants grow over the season, there is more carbon available for the soil organisms (Bardgett *et al.* 1997; Ilmarinen *et al.* 2005). Clearly, if the greatest abundance and diversity of soil organisms is desired from a sampling time, late summer-early autumn appear to yield the desired results.

Next steps

Recent reviews of our knowledge of soil systems have repeated the importance of simply knowing what is in the soil and what affects the distribution of soil organisms (Wall *et al.* 2005). We still do not have a solid understanding of how soil organisms interact, why they are where they are, and if there are any geographic patterns in soil biodiversity. Based on the results obtained in this study, it would appear that at the spatial scale used in this research the soil invertebrates are homogenous in space. That is to say, 200m was not a large enough distance to observe changes in the invertebrate assemblages. Future studies should aim at examining at what scale differences in the invertebrates can be discerned (500m? 1km? 10km?) and whether the differences are

related more to biome type than to small scale differences such as plant diversity. One of the first steps is to identify what organisms are in the soil in the first place, and this study contributes to the inventory of taxa that present in Alberta boreal aspen-parkland forest soils. Further research is needed to understand how soil organisms respond to change, and whether the temporal patterns I have observed in this study also occur in different places in Alberta, and are repeatable over longer periods of time.

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TABLES AND FIGURES

Table 2-1: t-test results for the average mean ranks for bacterial activity of single carbon source in Plot 1 versus Plot 2 (d.f. = 62, t-critical = 1.67, $\alpha = 0.05$).

	Plot 1	Plot 2
Mean	27.11694	37.88306
t-stat	-3.43953	
P value	<0.001	

Table 2-2: Measure of coarse woody debris for Plot 1 and Plot 2. The number of snags (stumps) and fallen logs are noted. Average decay stage is also listed on a scale of 1-7, (based on Stelfox, 1995).

	Plot 1	Plot 2
# snags	1	3
Average decay stage	6	6
# downed logs	2	12
Average decay stage	4	5

Table 2-3: PATN ANOSIM results for mean ranks for bacterial activity of single carbon source (BIOLOG) in Plots 1 & 2 analyzed by sampling time and plant species. Different superscript letters indicate significant differences between groups at $P<0.05$

Plot 1					
	Plant species	*Anemone*[a]	*Epilobium*[ab]	*Galium*[a]	*Vicia*[b]
	Sampling time	June[a]	July[a]	August[ab]	September[b]
Plot 2					
	Plant species	*Anemone*[a]	*Epilobium*[ab]	*Galium*[a]	*Vicia*[b]
	Sampling time	June[a]	July[a]	August[a]	September[a]

Table 2-4: Mean ranks for bacterial activity of single carbon source in Plots 1 & 2 grouped by plant species. (n = 8 for each plant species in each plot)

	Anemone canadensis	*Epilobium angustifolium*	*Galium boreale*	*Vicia americana*
Plot 1	14.17	18.92	11.52	21.39
Plot 2	13.66	18.04	13.18	21.12

Table 2-5: Analysis of similarity (ANOSIM) between sampling times over the 2004 field season for the invertebrate assemblages from both plots. Plots were grouped since there was no significant difference between them. Results were obtained using all organisms in the study identified to the finest taxonomic level. The p-value is shown.

	June	July	August
July	<0.0001		
August	<0.0001	<0.0001	
September	<0.0001	<0.0001	0.0160

Table 2-6: Analysis of similarity (ANOSIM) between sampling times over the 2004 field season. Results were obtained using nematodes identified to feeding type (microbivore, carnivore, plant parasitic) or larval stage. The p-value is shown.

	June	July	August
July	<0.0001		
August	<0.0001	<0.0001	
September	<0.0001	<0.0001	0.2342

Table 2-7: Analysis of similarity (ANOSIM) between sampling times over the 2004 field season. Results were obtained using the non-nematode taxa in the study identified to the finest taxonomic level. The p-value is shown.

	June	July	August
July	0.0080		
August	<0.0001	0.0160	
September	<0.0001	<0.0001	0.0180

Table 2-8: ANOVA results and Tukey's post hoc test for invertebrate abundance based on sampling time. The results for the two plots are combined as there was no significant difference between the plots. Post hoc test indicates which months are similar with $P<0.05$.

	p-value	June	July	August	September
Acari					
Oribatida	0.010	ab	a	ab	b
Astigmata	0.026	a	ab	ab	b
Mesostigmata	<0.001	a	a	b	b
Prostigmata	0.134	a	a	a	a
Endeostigmata	0.017	a	ab	ab	b
Collembola	0.004	a	a	ab	b
Tardigrada	<0.001	a	b	b	b
Nematodes					
Total	<0.001	a	b	b	b
Microbivores	<0.001	a	b	b	b
Carnivores	<0.001	a	b	c	c
Plant parasitic	<0.001	a	b	b	b
Larval	<0.001	a	b	b	b

Table 2-9: ANOVA results and Tukey's post hoc test for invertebrate taxon richness based on sampling time. Nematodes were excluded as they were only identified to functional group. Astigmata was excluded due to low richness (two life stages), while the Prostigmata and Endeostigmata were combined. The two plots were combined as there was no significant difference between the two. Post hoc test indicates which months are similar with $P<0.05$.

	p-value	June	July	August	September
All combined	<0.001	a	ab	b	c
Acari					
Oribatida	<0.001	a	a	ab	b
Mesostigmata	<0.001	a	a	ab	b
Prostigmata & Endeostigmata	0.031	a	ab	ab	b
Collembola	<0.001	ab	a	bc	c
Tardigrada	<0.001	a	b	b	b

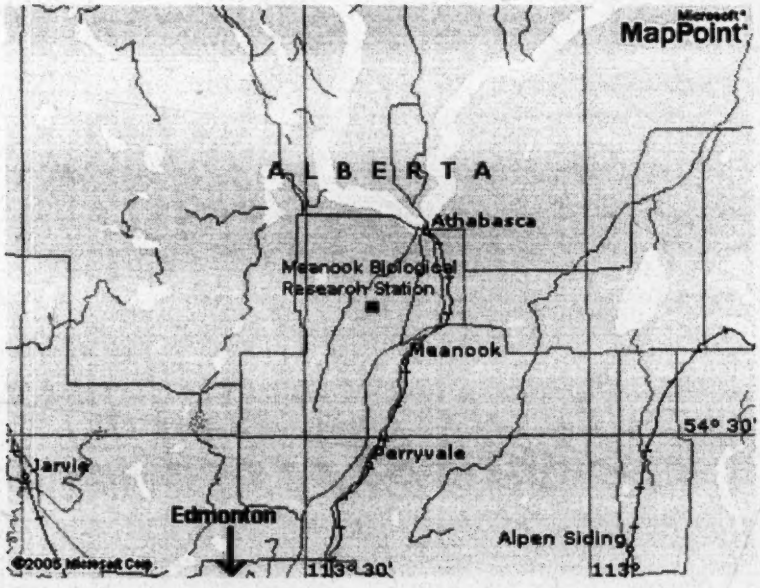

Figure 2-1 – Map of Alberta showing the location of the Meanook Biological Research Station

Figure 2-2 – Understory vegetation in Plot 2 at the Meanook Biological Research Station forest. Photo taken June 8, 2005.

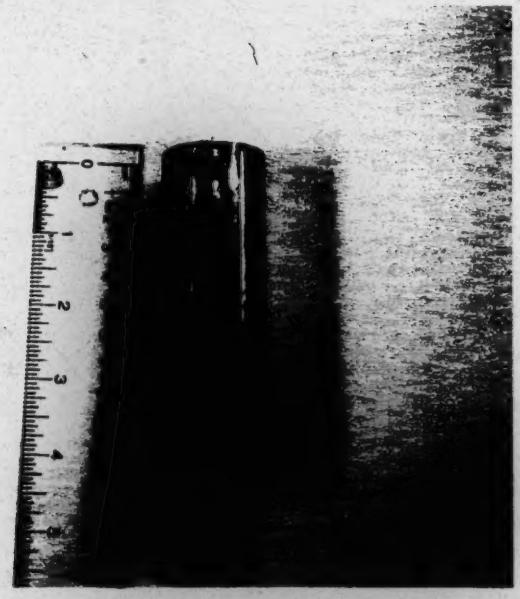

Figure 2-3 – Soil core used to sample the soil under the Meanook plants. Corer was inserted to the bottom of the hole, for a depth of 10 cm. The diameter of the corer is 2.5 cm.

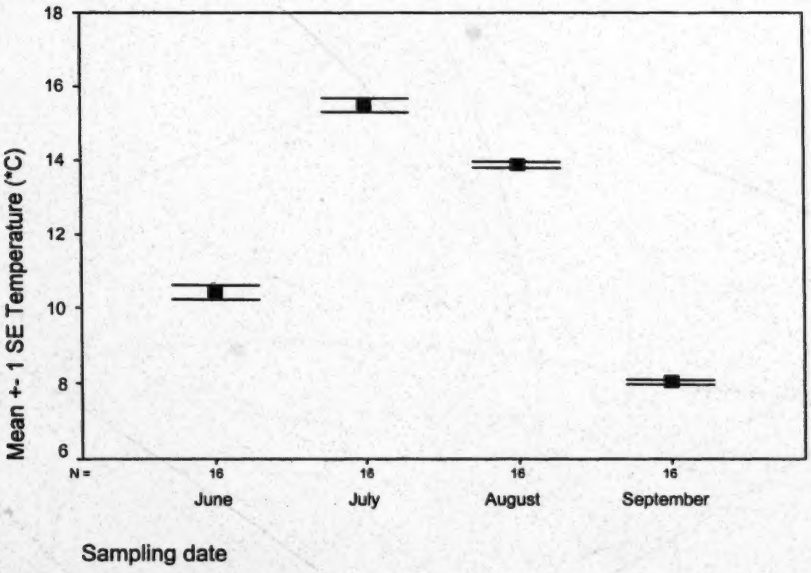

Figure 2-4: Mean soil temperature (°C) at the study plants during summer 2004, separated by sampling time.

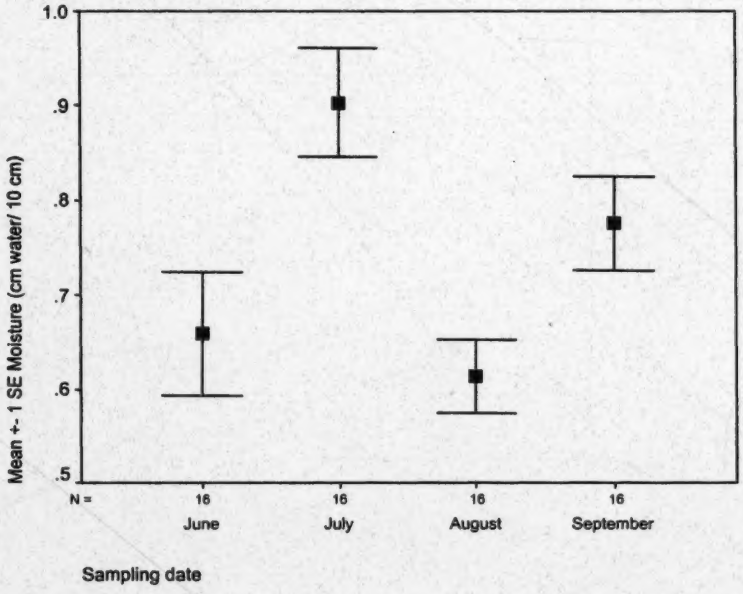

Figure 2-5: Mean soil moisture at the study plants in summer 2004, separated by sampling date. The moisture was recorded on a scale of 0 (very dry) to 10 (very wet) and converted to amount of soil water (cm) in the 10cm soil core.

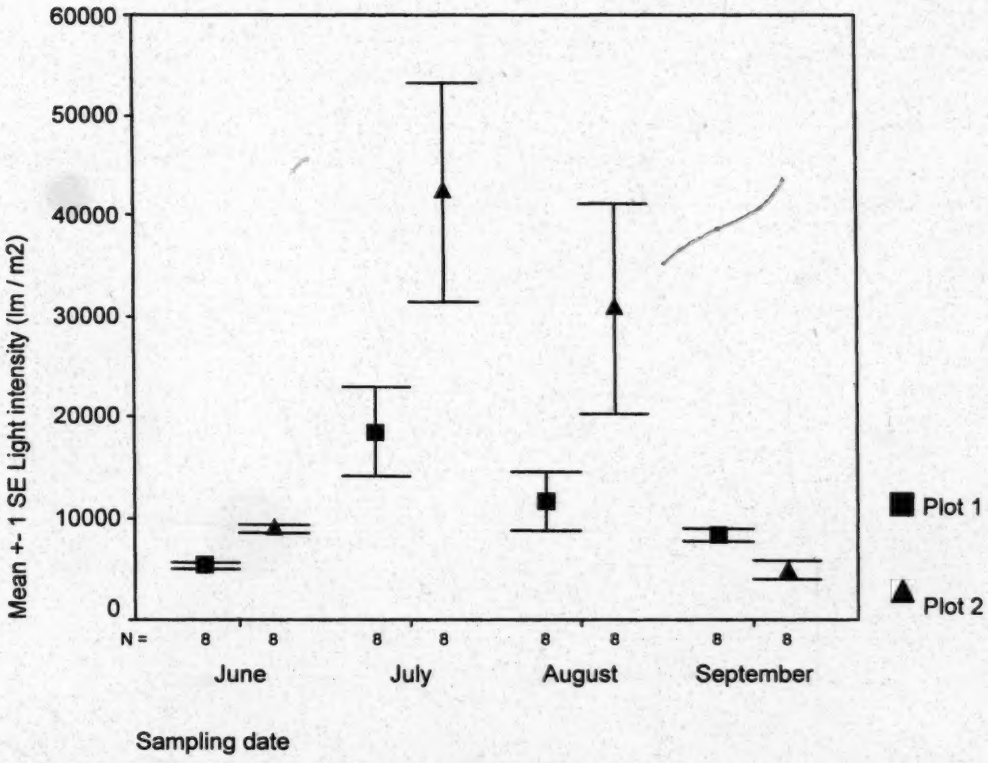

Figure 2-6: Mean light intensity in lm/m^2 at the study plants in summer 2004, separated by sampling time and grouped by plot.

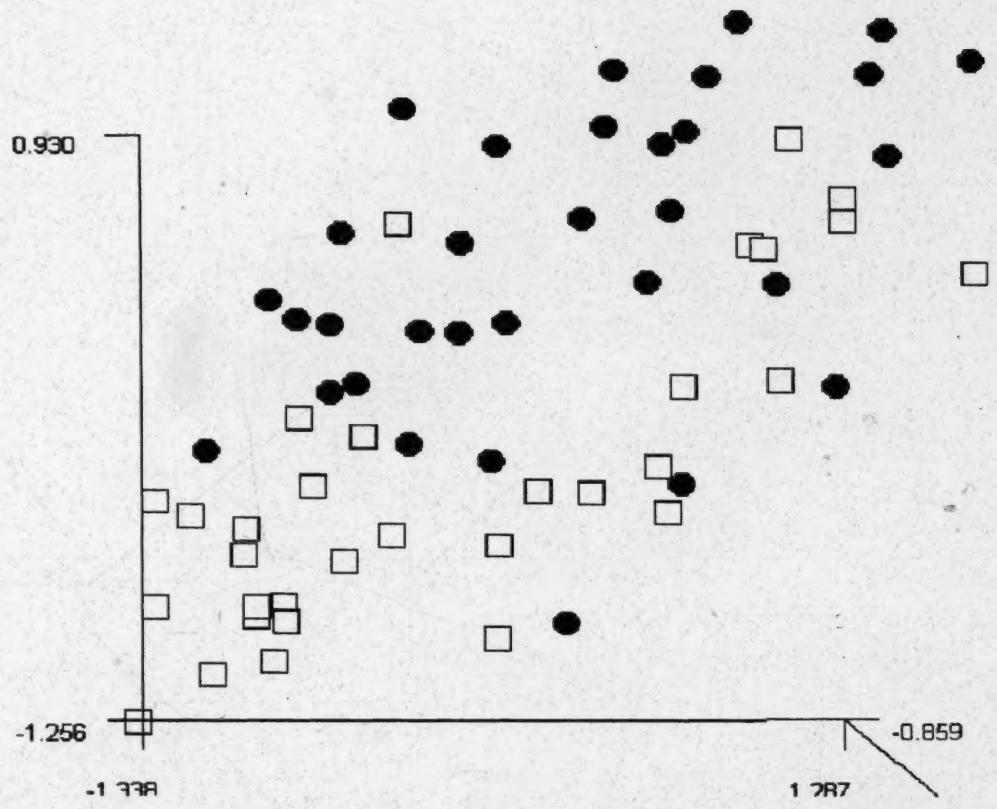

Figure 2-7: Bacterial community, based on rank of single carbon source utilization on a BIOLOG EcoPlate. The samples are separated by plot, Plot 1 is the open squares and Plot 2 in the black circles. The 3D ordination stress with a 1000 random starts was 0.1201.

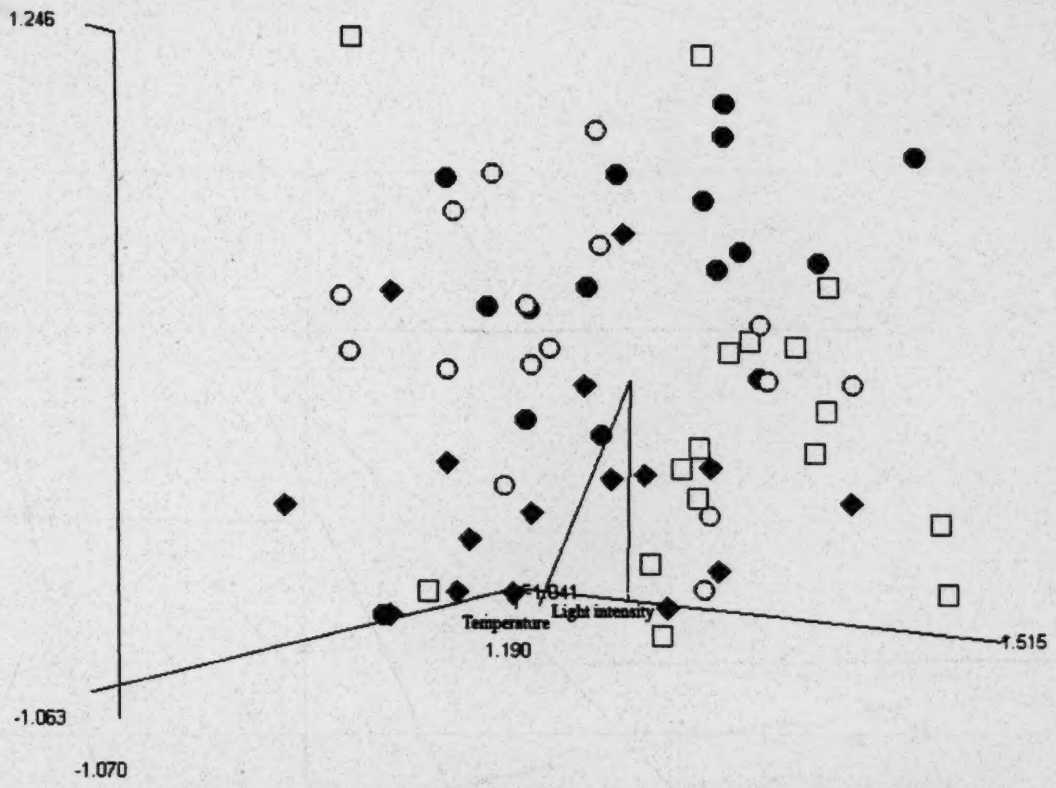

Figure 2-8: All Meanook invertebrates, identified to the finest level, separated by sampling time. June is represented by the open squares, July by the closed diamonds, August by the open circle and September by the closed circle. The 3D ordination stress for 1000 random starts was 0.1962. The vectors are abiotic factors that were identified by MCAO to be significantly correlated with the ordination at $P<0.05$.

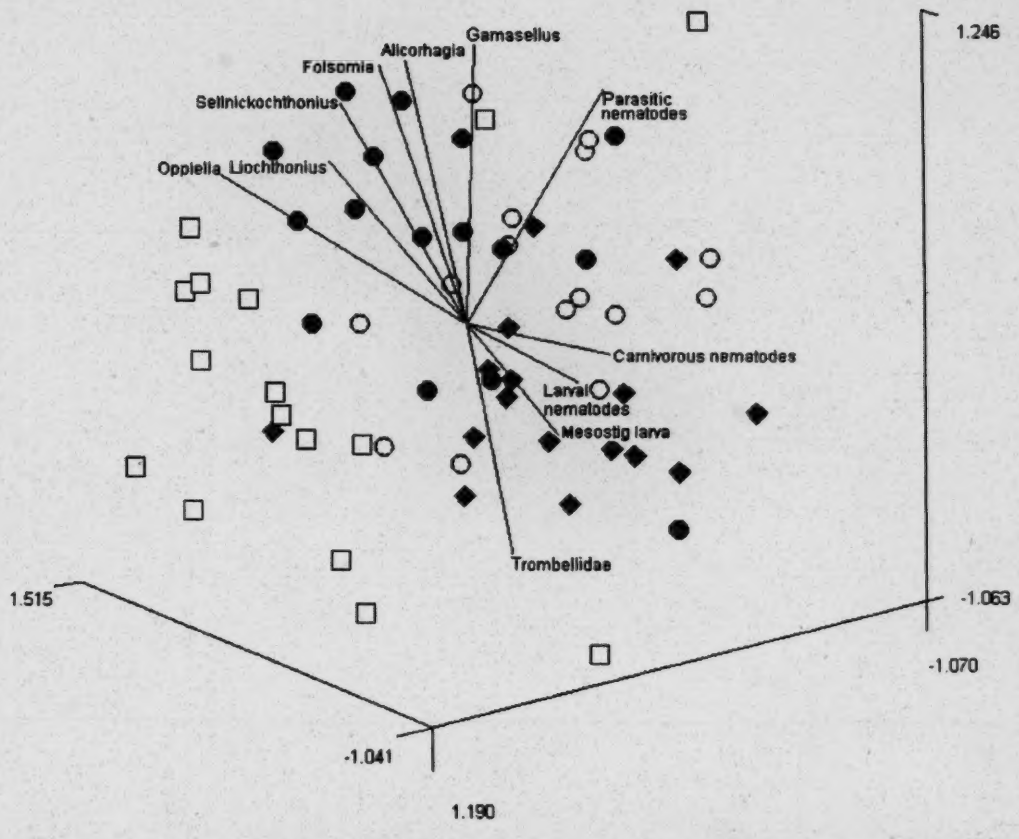

Figure 2-9: All Meanook invertebrates, identified to the finest level, separated by sampling time. June is represented by the open squares, July by the closed diamonds, August by the open circle and September by the closed circle. The 3D ordination stress for 1000 random starts was 0.1962. The vectors are invertebrate groups that were identified by MCAO to be significant at $P<0.01$ in creating the ordination.

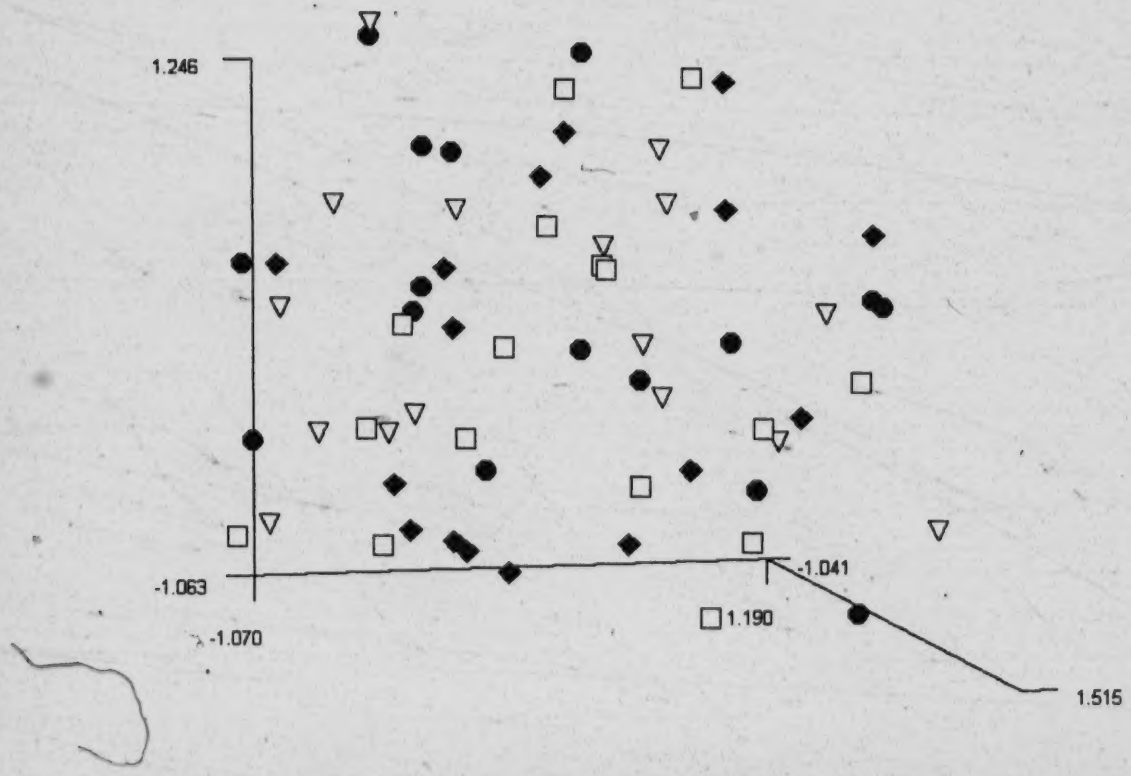

Figure 2-10: All Meanook invertebrates, identified to the finest level, separated by plant species. *Anemone* is represented by the open squares, *Epilobium* by the closed circles, *Galium* by the open triangles, and *Vicia* by the closed diamonds. The 3D ordination stress for 1000 random starts was 0.1962

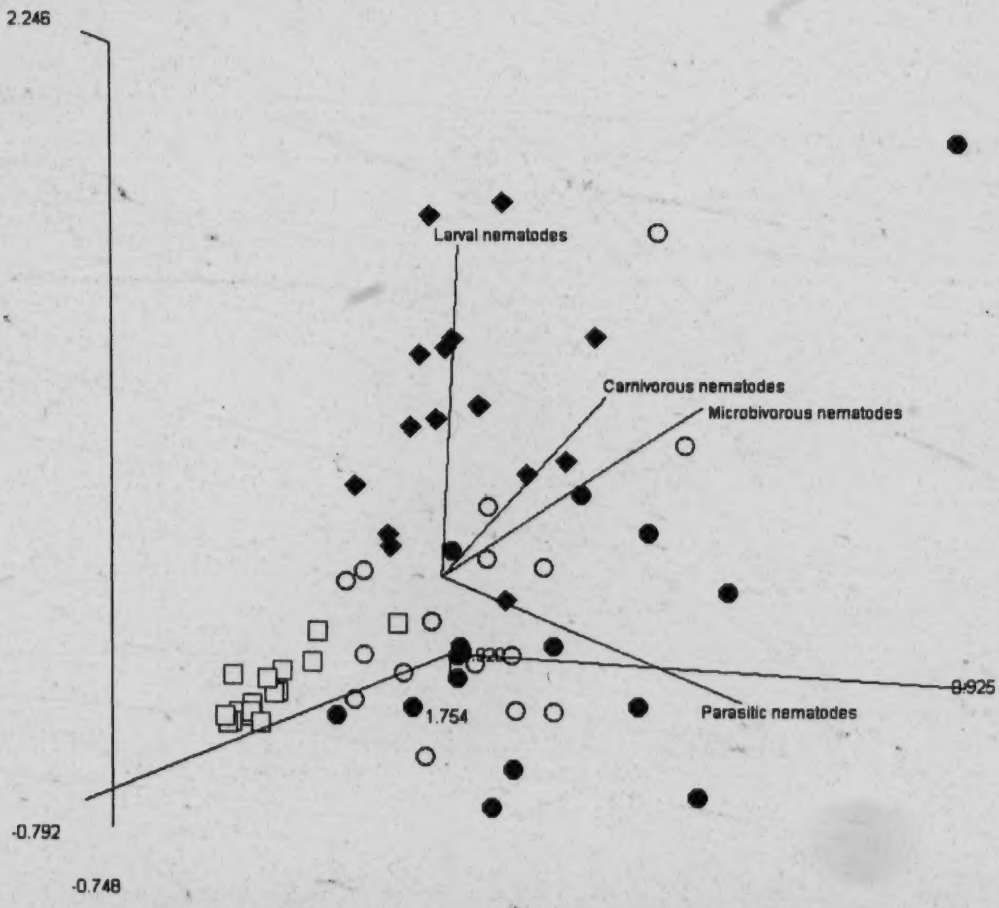

Figure 2-11: All Meanook nematodes, identified to either trophic grouping (Microbivore, Carnivore or Plant Parasitic) or Larval stage, separated by sampling time. June is represented by the open squares, July by the closed diamonds, August by the open circle and September by the closed circle. The vectors are significant at $P<0.05$ based on MCAO with 1000 random starts. The 3D ordination stress for 1000 random starts was 0.0603.

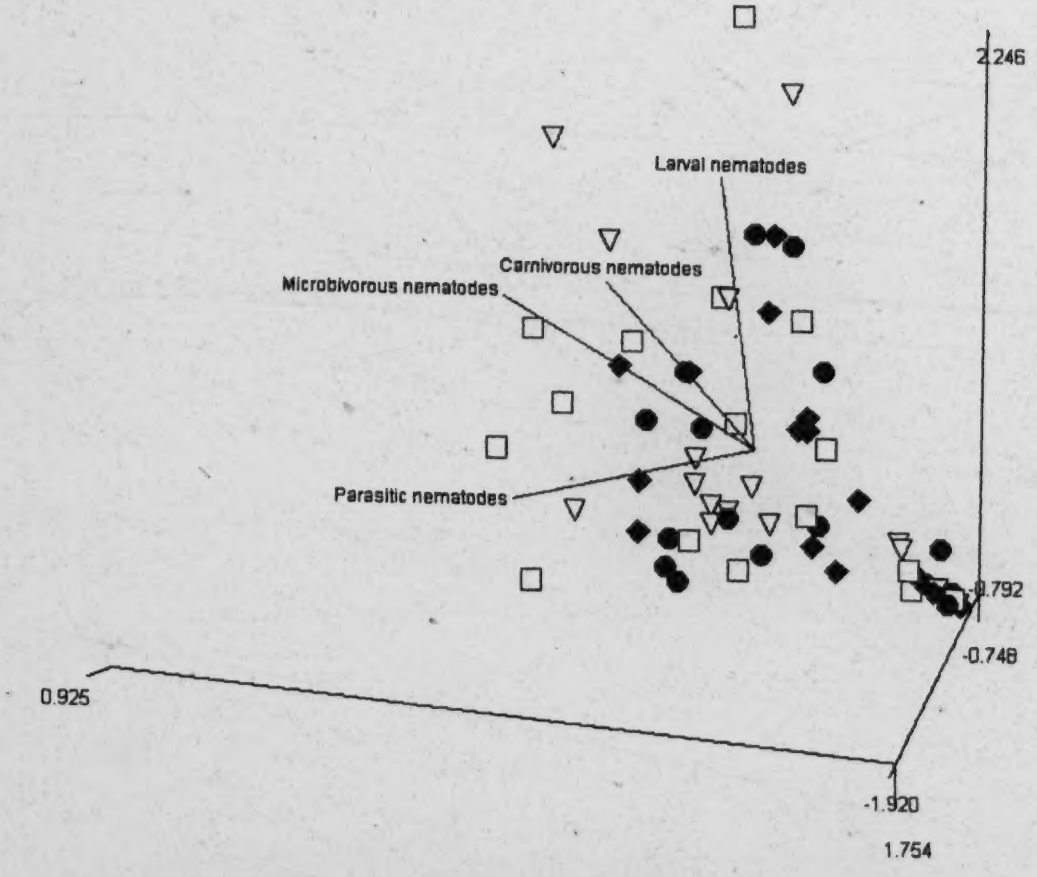

Figure 2-12: All Meanook nematodes, identified to either trophic grouping (Microbivore, Carnivore or Plant Parasitic) or Larval stage, separated by plant species. *Anemone* is represented by the open squares, *Epilobium* by the closed circles, *Galium* by the open triangles, and *Vicia* by the closed diamonds. The vectors are significant at $P<0.05$ based on MCAO with 1000 random starts. The 3D ordination stress for 1000 random starts was 0.0603.

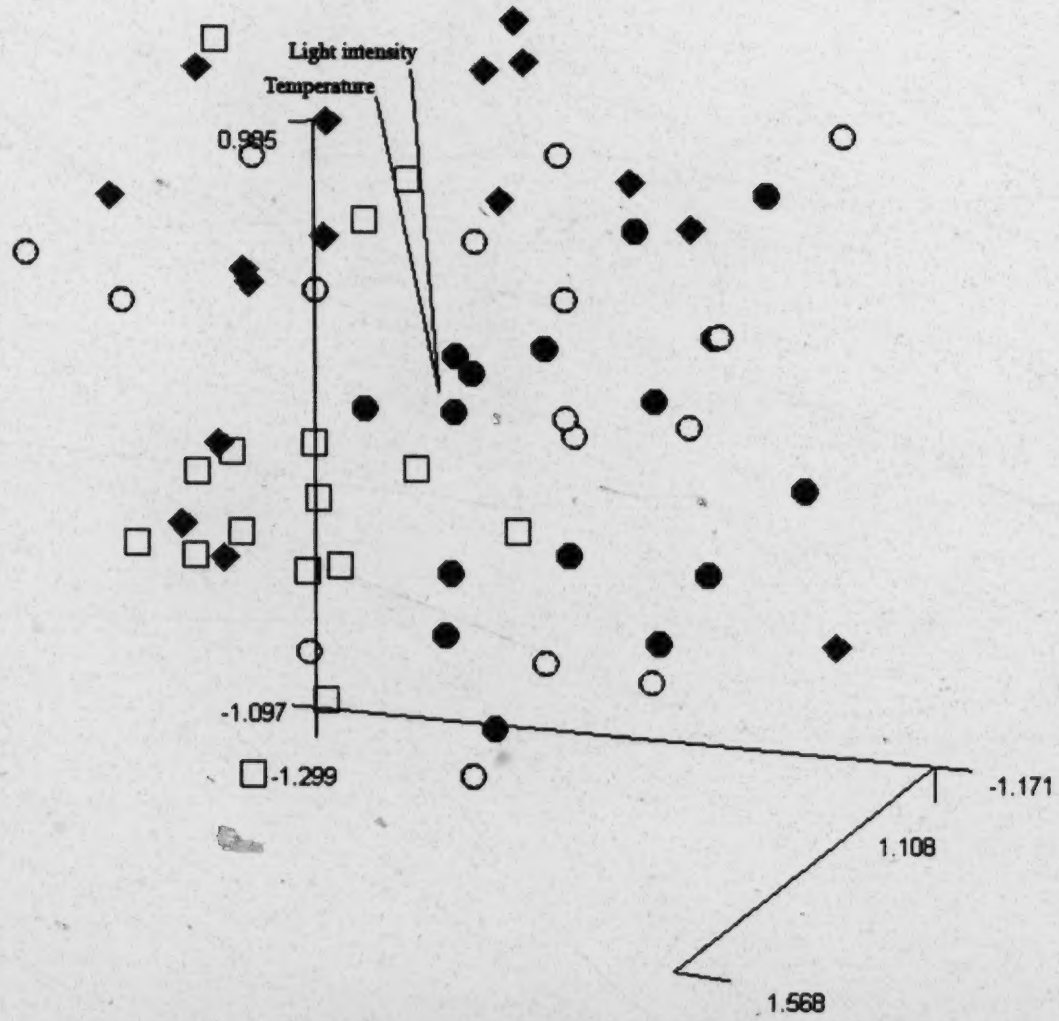

Figure 2-13: All Meanook non-nematode taxa, identified to the finest level, separated by sampling time. June is represented by the open squares, July by the closed diamonds, August by the open circle and September by the closed circle. The vector is significant at $P<0.05$ based on MCAO with 1000 random starts. The 3D ordination stress for 1000 random starts was 0.2203

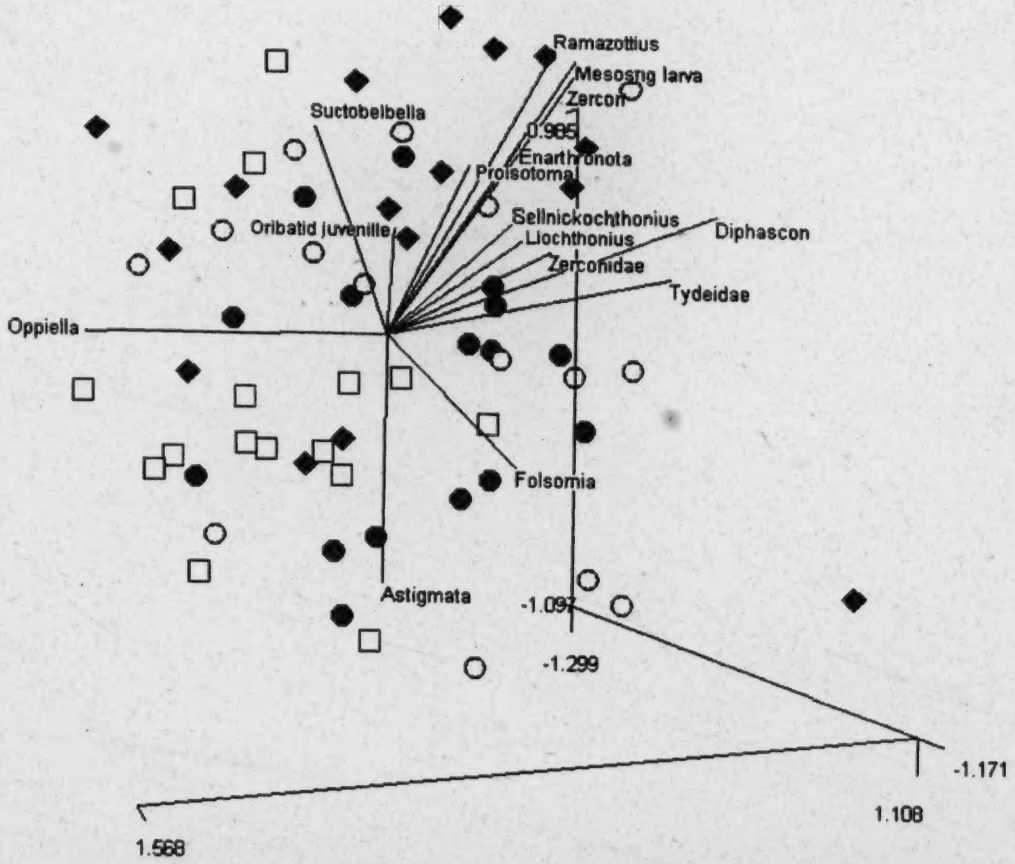

Figure 2-14: All Meanook non-nematode taxa, identified to the finest level, separated by sampling time. June is represented by the open squares, July by the closed diamonds, August by the open circle and September by the closed circle. The vectors are significant at $P<0.01$ based on MCAO with 1000 random starts. The 3D ordination stress for 1000 random starts was 0.2203

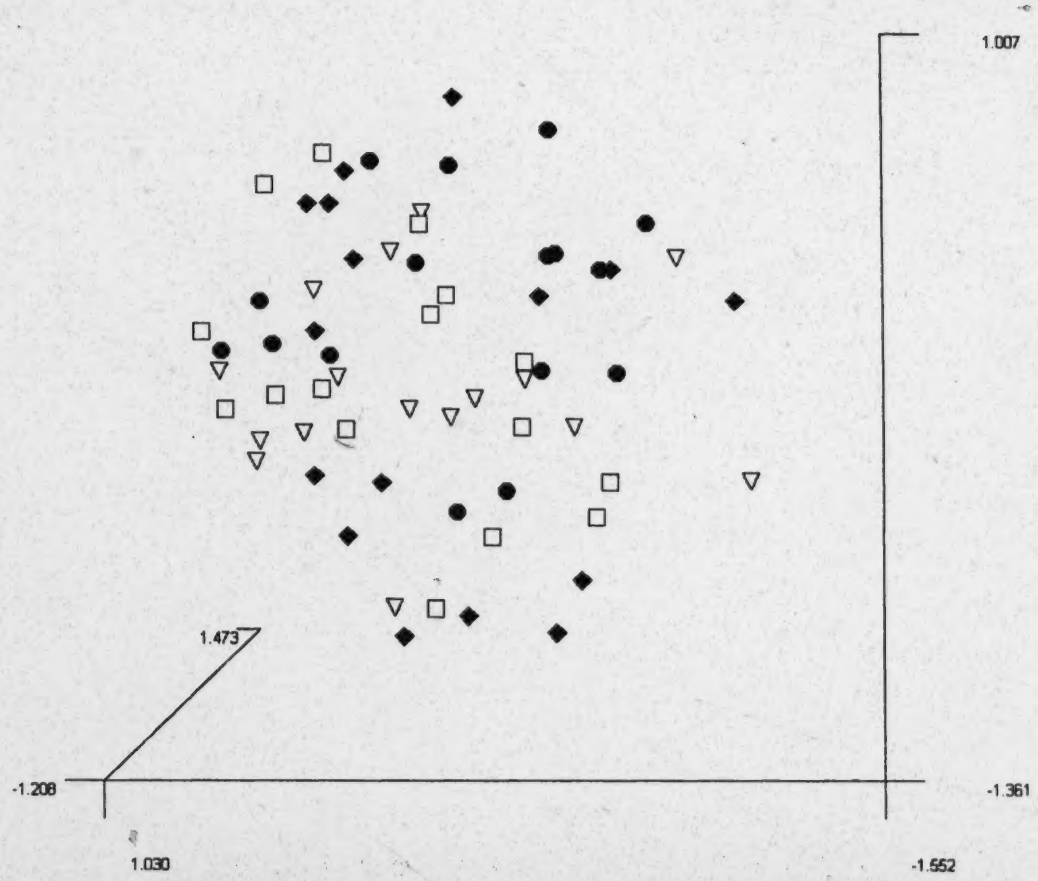

Figure 2-15: All Meanook non-nematode taxa, identified to the finest level, separated by plant species. *Anemone* is represented by the open squares, *Epilobium* by the closed circles, *Galium* by the open triangles, and *Vicia* by the closed diamonds. The 3D ordination stress for 1000 random starts was 0.2203

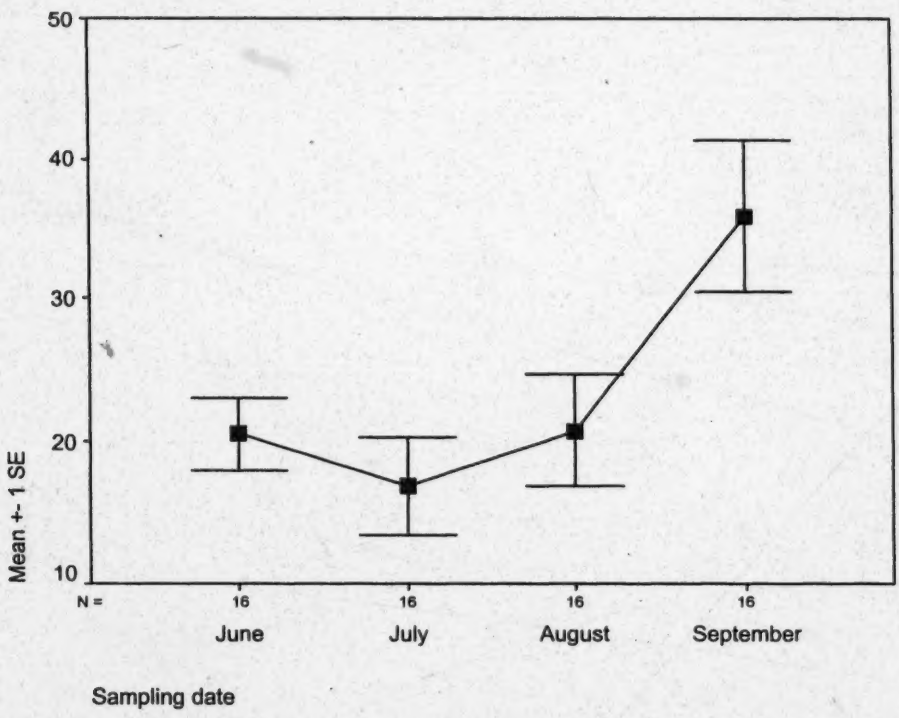

Figure 2-16: Mean oribatid abundance by sampling date. Data for the four plant species and the two plots have been pooled.

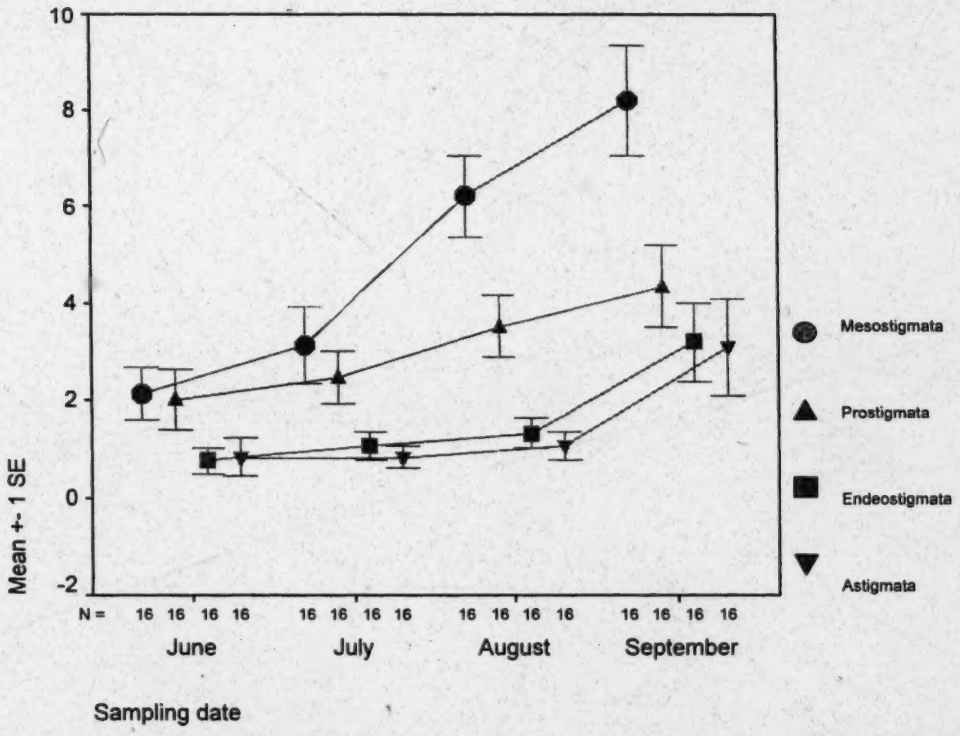

Figure 2-17: Mean mite (non-oribatid) abundance by sampling date. The Mesostigmata are represented by a circle, the Prostigmata by a triangle, the Endeostigmata by a square and the Astigmata by an inverted triangle. Data for the four plant species and the two plots have been pooled for each of the suborders.

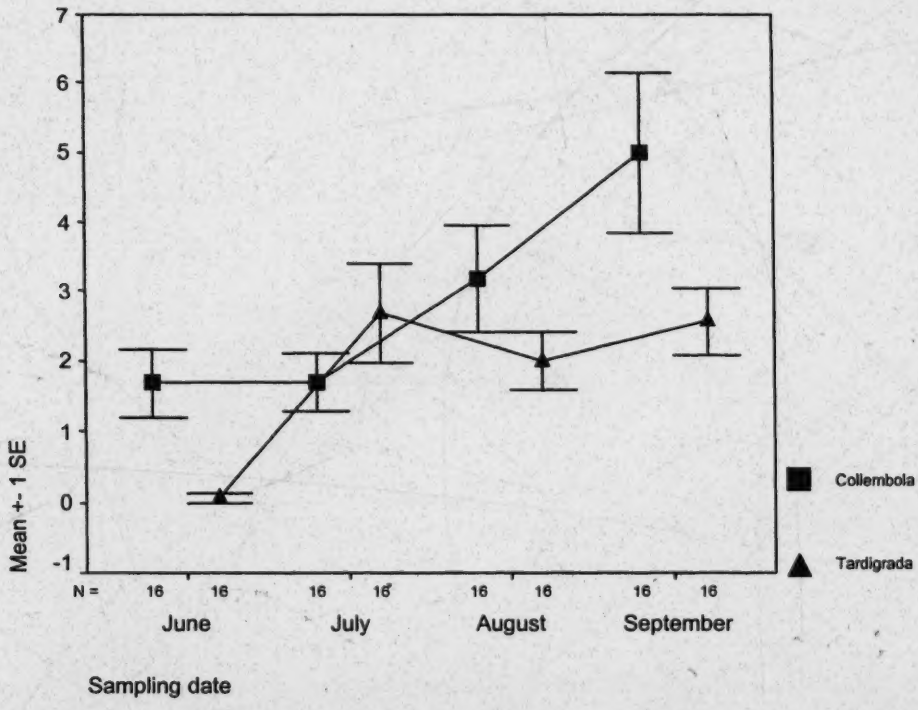

Figure 2-18: Mean collembolan and tardigrade abundance by sampling date. The Collembola are represented by the square and the tardigrades by the triangle. Data for the four plant species and the two plots have been pooled for each taxon.

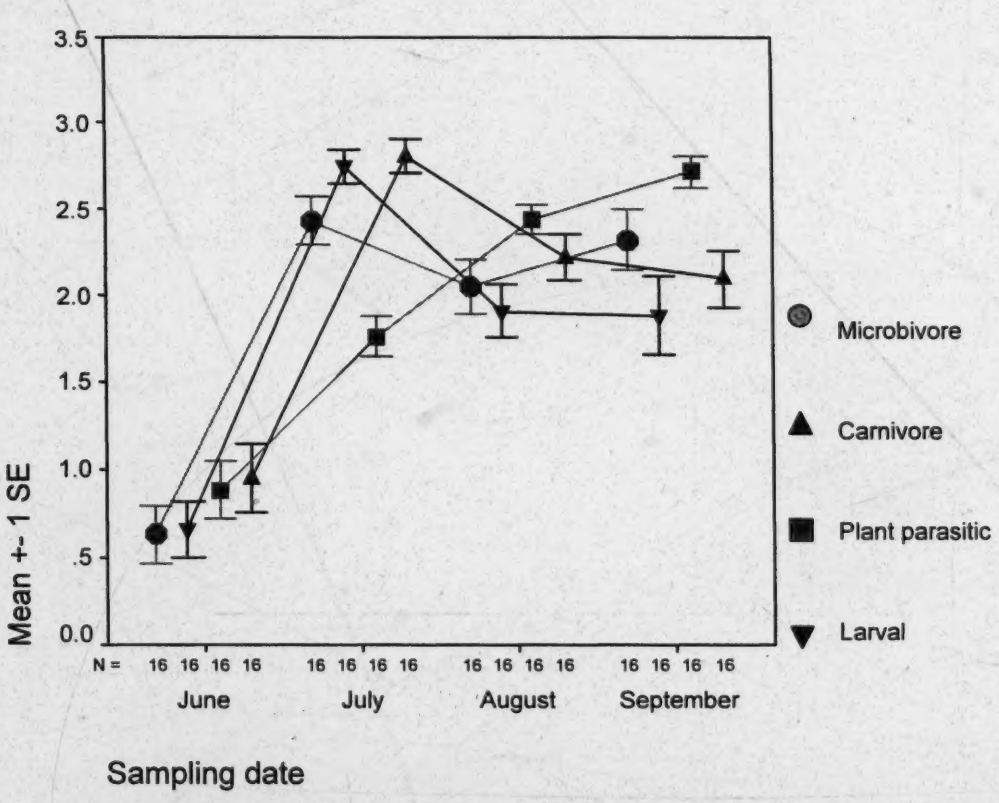

Figure 2-19: Mean nematode abundance by trophic group by sampling date. The microbivores are represented by the grey circle, the carnivore a black triangle, the plant parasite by a grey square and the larval nematodes by a black inverted triangle. Data for the four plant species and the two plots have been pooled for each taxa

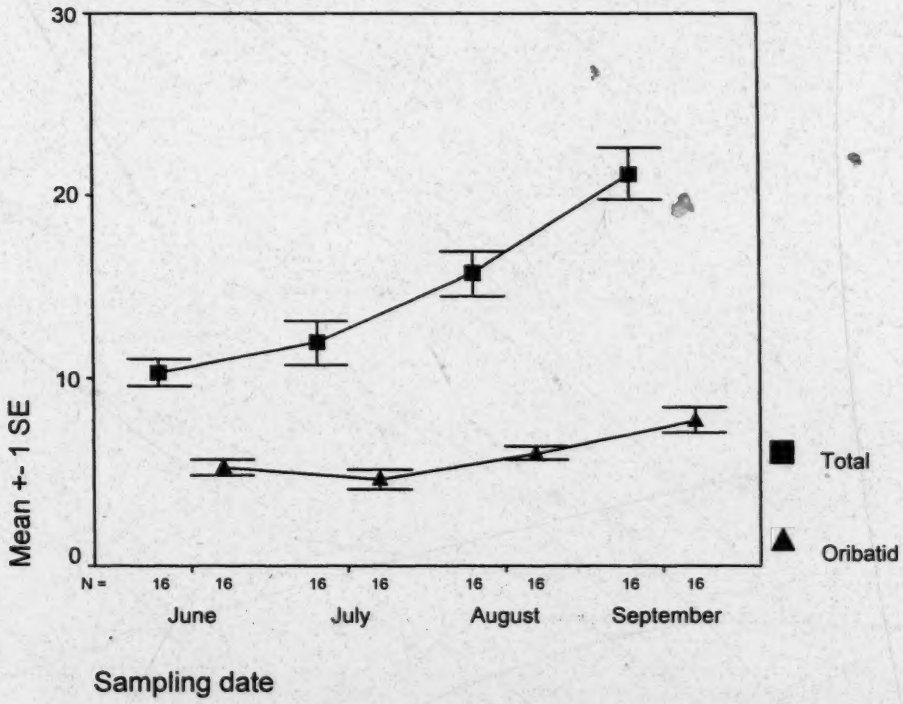

Figure 2-20: Taxon richness by sampling time; total taxon richness for all organisms is represented by the square, and taxon richness for the Oribatida is represented by the triangle. All samples at a given sampling time were pooled. Oribatids are included here as they were the most taxon rich group collected at Meanook.

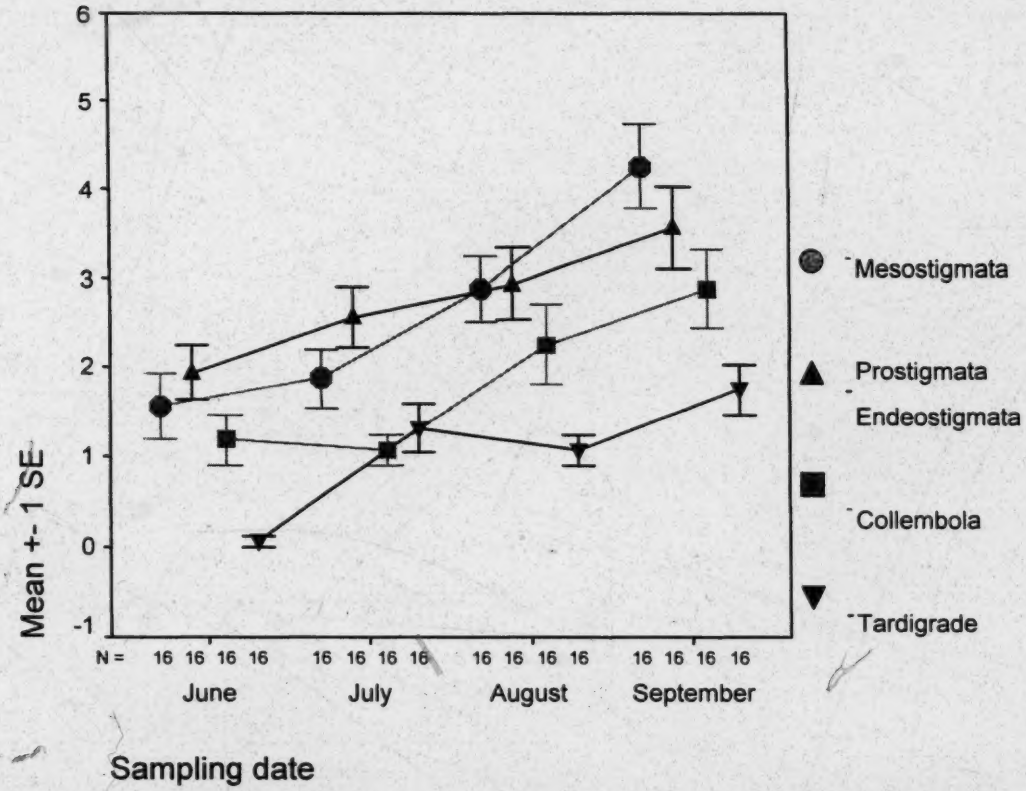

Figure 2-21: Taxon richness by sampling time. Total taxon richness for the Mesostigmata is represented by a grey circle, the Prostigmata/Endeostigmata by a black triangle, the Collembola by a grey square and the Tardigrada by a black inverted triangle. All samples at a given sampling time were pooled. To make this graph easily understood, Oribatida were included on Figure 2-20 as they were more taxonomically diverse then the other organisms.

THE EFFECTS OF SIMULATED ABOVE-GROUND AND BELOW-GROUND HERBIVORY ON INVERTEBRATE ASSEMBLAGES IN THE RHIZOSPHERE

INTRODUCTION

Historically, the above- and below-ground realms have been analyzed separately by terrestrial biologists, but lately there has been a growing understanding that these two areas are inextricably linked (Wardle 2002). This linkage occurs primarily through living plants, which simultaneously occupy both areas; the above-ground photosynthetic portion shunts sugars and other compounds below-ground, and the below-ground root system absorbs water and nutrients for use by the above-ground portion. In moving carbohydrates below-ground, some are released by the roots as exudates into the rhizosphere, the area immediately surrounding the roots (Grayston *et al.* 1996; Wardle 2002; Walker *et al.* 2003). These exudates contain both photosynthetic carbohydrates and other plant compounds that can affect other organisms in the soil (Grayston *et al.* 1996). Some exudates act as food sources for soil microbes (Wardle 2002), therefore indirectly affecting the soil fauna. The exudates can also act as agents of allelopathy, deterring growth of other plants in the vicinity (Bertin *et al.* 2003). Anti-microbial and anti-fungal compounds may also be released by the plant into the rhizosphere (Grayston *et al.* 1996; Walker *et al.* 2003). Anti-faunal compounds, that deter root pathogens such as root-feeding nematodes, can also be released into the rhizosphere (Topp *et al.* 1998). Thus, a plant has a strong influence over what organisms are present near its roots.

The quality and quantity of exudates released by an individual plant can be affected by its species, age, site conditions, nutritional status, pH, water availability, temperature, light intensity, microorganisms, and herbivory (Grayston *et al.* 1996).

Herbivory effect are not simply due to biomass removal as herbivory can cause an increase in the amount of carbon exuded from roots over the subsequent hours to a few days (Holland *et al.* 1996; Grayston *et al.* 1996). If herbivory persists, then carbon is shunted away from the roots to the shoots in order to maintain photosynthetic capabilities (Bardgett *et al.* 1998; Wardle 2002). Herbivory, both above- and below-ground, can induce production of defense compounds that function to deter future herbivore attacks (van Dam *et al.* 2003). These compounds are often made in the roots and so may be leaked out as part of the exudate material.

While it seems counterintuitive for an injured plant to release carbohydrates into the soil when they could be used to repair the above-ground damage from herbivory, it may actually be beneficial to the plant: by releasing the compounds, the plant can stimulate microbial activity in its rhizosphere which will increase nutrient availability (Grayston *et al.* 1996). This puts the plant is in a better position to repair the damage it has sustained.

Several studies have shown that above-ground herbivory results in a marked increase of organisms below ground, namely the bacterial-feeding nematodes (Ingham and Delting 1984; Seastedt *et* al. 1988; Bardgett *et al.* 1997; Wardle *et al.* 2004; Zelenev *et al.* 2004). This is likely due to increased carbon exudates being released by the roots in response to the herbivory (Holland *et al.* 1996) causing a subsequent increase in rhizosphere microbial biomass (Bardgett *et al.* 1998). Herbivory on the below-ground plant parts can also influence the soil community (Grayston *et al.* 2001). While root herbivory can cause an increase in the amount of exudates being released through the process described above, mechanical damage to the roots may also cause roots to become

'leaky' (Yeates *et al.* 1998). Below-ground herbivory has also been shown to increase the biomass of microbes present in the rhizosphere (Yeates *et al.* 1998; Denton *et al.* 1999; Grayston *et al.* 2001). While the effects of both types of herbivory on the soil community have been examined individually, only a few studies have looked at the combined effects of above- and below-ground herbivory on soil biota (Bezemer *et al.* 2003; van Dam *et al.* 2003).

Studies on the effects of defoliation on soil biota have generally focused on grassland systems or on a small number of herbaceous plant species typical of open habitats, primarily *Trifolium repens* L. (Mawdsley *et al.* 1997; Denton *et al.* 1999; Wardle *et al.* 1999; Mikola *et al.* 2001; Ilmarinen *et al.* 2005; Mikola *et al.* 2005), and *Plantago lanceolata* L. (Mikola *et al.* 2001; Ilmarinen *et al.* 2005; Mikola *et al.* 2005). Forest systems, and their associated understory vegetation, have not been examined. In this study, I use two species of herbaceous boreal-forest plants (*Epilobium angustifolium* L. and *Oenothera biennis* L.) to test the effects of above- and below-ground herbivory on the rhizosphere microbes and invertebrates. The invertebrates I examined were Nematoda, Acari (mites), Collembola (springtails) and Tardigrada. I predicted a bottom-up effect of artificial herbivory: an increase in carbon exudation would occur after herbivory and this would result in an increase in the biomass of microbes, followed by that of microbivorous animals, and finally by carnivorous animals. I also examined whether the rhizosphere community responded differently to damage to leaves vs. roots, or to old vs. young leaves.

METHODS

Growth and preparation of plants – 2004

Two boreal herbaceous plants were chosen for this experiment: yellow evening primrose (*Oenothera biennis* L.) and fireweed (*Epilobium angustifolium* L.). It was desired to have two boreal herbaceous plants, and these two were chosen based on seed availability in the University of Alberta Department of Biological Sciences greenhouse. On May 18th and 19th 2004, seeds were planted in 8" pots of pasteurized potting soil (25% topsoil, 35% perlite, 40% peat moss), 20 pots for each species. The seeds were obtained from the University of Alberta Department of Biological Sciences greenhouse. The soil was pasteurized (temperature >400°F for 6 hours, Lansa soil pasteurizer, Johnsen Machine Company Ltd.) to remove any organisms that may have been initially present in the potting soil. Potted seeds were placed in the Biological Sciences greenhouse at the University of Alberta, and allowed to grow. Plants were watered three times a week, more if the weather was warm and the soil was drying out. On June 10th 2004, soil was collected from the forest at the Meanook Biological Research Station (54° 37' N, 113° 21' W) near Athabasca, Alberta, and brought back to the lab. The soil was sieved through a 4 mm sieve to remove large debris such as twigs and roots, while still allowing the organisms to remain. On June 14th, sieved forest soil mixed with perlite was added to 18 pots. Perlite was added to create pore space in the soil, and to prevent compaction due to lack of structure caused by sieving out anything larger than 4 mm. I transplanted three greenhouse-grown plants to each forest-soil pot to ensure at least one would survive. Nine pots per species were created. After about two weeks when the plants had established, I selected the largest individual per pot and removed other two. At this point,

there were 17 pots per species (Fig. 3-1): 8 control pots with pasteurized soil and 9 experimental pots with Meanook soil, each containing only one plant.

Effect of herbivory on soil carbon – 2004

This experiment tested for increases in carbohydrate exudates as a result of herbivory using plants in pasteurized soil (microbes might otherwise immediately consume the extra carbon). For each plant species (primrose and fireweed), the eight pots with pasteurized soil were split into two treatment groups: four root-and-leaf herbivorized pots (Root and Leaf) and four root-herbivorized pots (Root Only). Initially, the treatments were leaf herbivory versus a control, however once the results from the 2004 experiment were obtained (see *Soil carbon* section in Results), it became clear that the soil cores themselves were damaging the roots. It was then decided to call this 'simulated root herbivory'. The Root Only pots had two 2.5 x 5 cm cores taken through the root mass, 48 hours apart; the first core simulated herbivory, and the second allowed for measurement of response to that damage. For the Root and Leaf pots, the 'root herbivory' soil core was taken 24 hours after leaf clipping occurred and the second core was taken 24 hours later. The first soil cores were taken on August 4th. To extract the soluble carbon, a surrogate for root exudates, 10 g wet weight of soil was taken from the cores and mixed with 50 mL of distilled, de-ionized water filtered to 0.22 μm (Millipore Corporation). The mixture was shaken for one hour on a mechanical shaker and then filtered through Whatman 40 filter paper (pore size 8.5 μm). The filtrate was then frozen until the analysis could take place. The remaining cored soil was weighed, dried for 12 hours at 90°C, and weighed again to determine the moisture content of each sample. On

August 5th, 24 hours after the cores were taken, plant growth was characterized by measuring the height of the plant, number of leaves, average length on leaves (primrose) or branches (fireweed) and for fireweed only, the number of branches was counted. Following this, 60% of leaves of the Root and Leaf plants were removed by clipping. Leaves from the primrose were removed from the outermost whorl (oldest) towards the middle, while fireweed leaves were removed from the top (youngest) downwards (see *Effect of leaf age at clipping on soil microbial assemblages – 2005* for a test of the effects of leaf age on soil microbes). On August 6th, 24 hours after defoliation (48 hours after root herbivory), I again took cores from the pasteurized soil pots. The carbon was extracted in the same way as stated above. On October 4th, the samples were removed from the freezer and allowed to thaw. On October 5th, the samples were analyzed for soluble carbon content using TOC-V_{CSN} (total organic carbon analyzer), Shimadzu Scientific Instruments.

The correction factor for moisture content (MC) of the samples was determined by the equation:

$$MC = \frac{\text{(wet weight of soil + bag)} - \text{(dry weight of soil + bag)}}{\text{(dry weight of soil + bag)} - \text{(dry weight of bag)}}$$

The corrected dry weight (DW) of the soil was then determined through the equation:

$$DW = 10 / (MC + 1)$$

where 10 was the amount of wet weight soil (g) used in the analysis. The soluble carbon results (in ppm) were then multiplied by 50 to convert to μg carbon, then are divided by the DW (in g) to determine the amount of carbon in μg per gram of dry soil, which was used for statistical analysis.

Effect of herbivory on soil carbon – 2005

Due to concerns with the results obtained in the summer of 2004 (see Results, *Soil carbon*), it was decided to repeat the experiment, in part, in the summer of 2005. The same two species of plants were used, fireweed and primrose. On May 4th 2005, seeds were planted in 8" pots of pasteurized potting soil (25% topsoil, 35% perlite, 40% peat moss), 18 pots for each species. The procedure for plant germination in 2004 was repeated in 2005. Due to difficulty in germination (some seeds did not germinate), there were only 15 pots of fireweed while primrose retained the original 18. The sampling design for this experiment involved three treatment groups: Root Only pots (cored at Day 0 and Day 2), Control pots (cored only at Day 2), and Leaf Only pots (clipped at Day 1 and cored Day 2). The pots were split equally among the treatments; primrose had 6 in each treatment and fireweed had 5 in each treatment.

On July 26th 2005, plant measurements were taken following the same procedure as for 2004. At this time, one core (2.5 x 5 cm) was taken from each pot of the Root Only pots; this is Day 0. After 24 hours (Day 1), 60% of the leaves were removed from the Leaf Only pots; older leaves from primrose and younger leaves from fireweed, as per the 2004 protocol. After another 24 hours (Day 2), all pots had a core taken. The soluble carbon was extracted the same as above with a few modifications: the filter paper used

was Whatman 42 (pore size 2.5 μm) and the samples were in the freezer for a shorter amount of time. Samples were removed from the freezer August 7th 2005 and analyzed August 8th 2005.

A further test of the soluble carbon was done on September 13th 2005 using a common greenhouse plant, *Pilea cadierei* Gagnep. & Guillaum (aluminum plant). This plant was chosen based on availability in the greenhouse and the fact that there was a sufficient number of single pots to sample. Ten pots were made available in the Biological Sciences greenhouse and on September 13th five pots were cored (2.5 x 5 cm). After 48 hours all ten pots were cored and these cores were analyzed for soluble carbon in the same way as above, using Whatman 40 filter paper (same as for 2004). This test was done to verify the effects of root coring on the soluble carbon in the soil with those being cored twice the Root Only treatment, while those cores only once acting as the Control.

Effect of leaf age at clipping on soil microbial assemblages – 2005

Here I tested whether clipping old or young leaves had any effect on soil microbial assemblages as potentially mediated by differences in exudate quality or quantity. Phospho-lipid fatty-acid (PLFA) analysis was performed to determine differences in the microbial assemblages as well as differences in biomass. Phospholipids are components of the membranes of living cells, and microbes also contain fatty acids in their membranes (Zelles 1999). PLFA analysis allows both bacteria and fungi to be tested. This analysis identifies the different PLFAs that are present in a sample, which can be used to assess the assemblage difference between samples, as well

as the differences in microbial abundance (Zelles 1999). Due to costs associated with this analysis, it was performed only on fireweed. On May 5th 2005, seeds were planted in 8" pots of pasteurized potting soil (25% topsoil, 35% perlite, 40% peat moss), 18 pots for each species. The seeds were obtained from the University of Alberta Department of Biological Sciences greenhouse. The procedure for plant germination in 2004 was repeated in 2005. On June 8th 2005, soil was collected from the forest at the Meanook Biological Research Station (54° 37' N, 113° 21' W) near Athabasca, Alberta, and brought back to the lab. The soil was sieved through a 4 mm sieve to remove large debris such as twigs and roots, while still allowing the organisms to remain. On June 10th, sieved soil mixed with perlite was added to 20 pots. Two plants were transplanted from the pasteurized soil into the Meanook soil pots to ensure at least one would survive. After about two weeks, one was removed so there was only one plant per pot.

The sampling design had four treatments: Control (no clipping), Young (only young leaves removed), Old (only old leaves removed) and Both (a mix of young and old leaves removed). On August 9th, plant measurements were taken using the same procedures as for the soluble carbon experiments, and 60% of the total number of plant leaves were removed from the Young, Old, and Both plants. For Both, 30% removal was of young leaves and 30% removal was of old leaves. On August 11th, one soil core (2.5 x 5 cm) was taken per pot and was placed in the -80°C freezer until analysis could take place.

On October 19th, the samples were removed from the freezer and analyzed. From each sample, two sub-samples were taken for analysis; 1.5 g dry weight of soil was used for each sub-sample. After extraction and liquid fractionation, each sample was entered

into a gas chromatographer to analyze the phospho-lipids and fatty acids. This was done by Dr. Sylvie Quideau's lab (Department of Renewable Resources, University of Alberta). The results obtained are in percent PLFA, (ie. the percentage each PLFA represents per sample). Before analysis can take place, the data must be converted into nmol/g dry soil. This is done by the following equation:

$$\text{nmol/g dry soil} = 10^9 * (((\%\ PLFA_a / \%\ 19{:}0) * [19{:}0] * \text{vol. } 19{:}0) / \text{dry mass of soil})$$

where [19:0] is 0.00008 mol/L, vol. 19:0 is 0.0002 L, the dry mass of soil is 1.5 g, % $PLFA_a$ is the PLFA of interest (obtained from the analysis), and % 19:0 is the standard entered into the analysis and is part of the output (White and Ringelberg 1998).

Once the data were converted, I removed all columns that represented PLFAs not present in the samples and those columns (PLFA) with only 1 observation. The PLFA 19:0 was removed as it was the standard, and any PLFA's above 20 carbon chains long were removed as these are non-fungal eukaryote PLFAs, mostly protozoa and fine roots (White and Ringelberg 1998). To avoid pseudo-replication, the average of the two sub-samples was taken for each of the samples and this was used for statistical analysis. To look at the abundance of the microbes, the sum of all PLFAs is added together to get the total amount of microbial biomass in nmol/g dry soil. The PLFA's were also assigned to groups based on whether they were indicative of gram positive bacteria, gram negative bacteria, or fungi as per Hassett and Zak (2005).

Effects of herbivory on invertebrate assemblages – 2004

This experiment tested the effect of simulated above- and below-ground herbivory on the soil invertebrate assemblages using plants in soil brought back form the Meanook Biological Research Station. For each plant species (primrose and fireweed), the nine pots with Meanook soil were split into two treatment groups: five root-and-leaf herbivorized pots (Root and Leaf) and four root-herbivorized pots (Root Only). On August 5th, plant growth characters were noted (same as for the soluble carbon experiments) and each pot had 2 cores (2.5 cm x 5 cm) taken to analyze the baseline invertebrate assemblage structure (Fig. 3-2). This coring was also the simulated root herbivory. This was designated Day 0. Once the cores were taken, 60% of the Root and Leaf plants' leaves were removed by clipping. Leaves from the primrose were removed from the outermost whorl (oldest) towards the middle, while fireweed leaves were removed from the top (youngest) downwards. When the cores were taken, the holes were refilled using pasteurized soil from an extra pot of the same plant species. Core locations were noted and not re-sampled.

To see if herbivory (and presumably increased exudation) stimulated nematode egg hatching, cores were taken again on August 9th, Day 4 after simulated herbivory. Two more sampling time occurred 20 and 40 days after defoliation, on August 25th, Day 20, and September 14th, Day 40. Each time, the two cores were mixed in the bag and 25 mL was used for Baermann funnel extraction of nematodes and tardigrades. The Baermann funnel is a live-extraction method that uses a funnel with a plastic tube clamped at the end. The sample is placed on a wire mesh in the funnel and water is added to cover the sample. The active nematodes and tardigrades then swim/crawl out of

the soil and collect in the bottom of the plastic tubing (Dindal 1990). I made some modifications to the standard method: the sample was wrapped in cheesecloth and placed directly in the funnel, and the funnel was placed in a collecting jar instead of tubing. For extraction of the microarthropods I used the Berlese/Tullgren (Dindal 1990). The soil sample was placed in on cheesecloth on a coarse mesh in a large funnel. At the bottom of the funnel was a collecting jar with 60% ethanol; a 40 watt light bulb above the funnel was used to desiccate the soil sample and cause the arthropods to travel downwards until they fell into the collecting jar.

The Baermann funnels were run for three days (Dindal 1990) with the jars stored in the fridge until sorted. Due to a problem with the refrigerator, the nematodes and tardigrades for Day 40 were frozen and partially disintegrated upon melting, and so accurate counts were not possible. Therefore, for the nematodes and tardigrades there are data only for Day 0, Day 4 and Day 20, while the remaining invertebrates also have data from Day 40. I sorted the live nematodes and tardigrades with the aid of a dissecting microscope. Nematodes were fixed with hot FA 4:1 (formalin: alcohol, 4:1) and mounted either in glycerin or Bio Quip #6371 PVA (lactic acid, phenol and polyvinyl alcohol). The tardigrades were mounted in PVA. The Berlese/Tullgren funnels were run for 4 days, all arthropods were fixed in 60% ethanol, sorted and mounted in PVA for identification. Nematodes large enough to have identifiable mouthparts were assigned to trophic groupings (microbivore, carnivore, and plant parasitic); juveniles with unidentifiable mouthparts were categorized simply as 'larvae'. The tardigrades (Ramazzotti and Maucci 1983; Dastych 1988), collembolans (Christiansen and Bellinger 1981), oribatid mites (Balogh 1961; Balogh 1963; Balogh 1965; Balogh and Mahunka

1983; Subias and Balogh 1989) and the mesostigmatan mites (Walter unpublished key 2004) were identified to genera, while the remaining mites were identified to family (Walter and Proctor 2001) due to lack of identification keys beyond that level. The arthropods and tardigrades were also assigned to trophic groups for analysis: phytophages (plant eaters), microbivores (microbe eaters), omnivores, and carnivores. The Prostigmata mite families and the Endeostigmata were verified by Dr. Heather Proctor (University of Alberta), the Oribatida were verified by Dr. Dave Walter (University of Alberta), Dr. Roy Norton (SUNY College of Environmental Science & Forestry) and Derrick Kanashiro (Alberta Research Council), the Mesostigmata were verified by Dr. Dave Walter, the Tardigrada were identified by Matthew Boeckner (Ph.D. candidate, University of Alberta), and the Collembola were verified by Dr. Jeff Battigelli (Earthworks Research Group). Assistance with placing the nematodes into trophic groups was provided by Matthew Boeckner.

Statistical analysis

Soluble carbon results for 2004 were analyzed using repeated-measures ANOVA in SPSS 11.5 (SPSS 2002). The amount of carbon (ppm per g dry soil) was the within-sample variable while treatment and plant species were the between-sample factors. For the 2005 results, the Root Only pots, as they were cored twice (Day 0 and Day 2), were analyzed using a repeated-measures ANOVA as above. For all the Day 2 results from 2005, the treatments were compared using univariate ANOVA with treatment and species as the between-subject variables. For the *Pilea* pots, the Root Only and Control pots were compared with a t-test in SPSS 11.5.

I compared PLFA data on the soil microbial assemblages using ssh-MDS with PATN 3.03 (Belbin 1989), a semi-strong hybrid, multi-dimensional scaling technique. The Gower metric was used for the analysis. This was done on the raw PLFA as nmol/g soil data. Analysis of similarity (ANOSIM) was performed comparing the different treatments of leaf age (Control, Old, Young, and Both) on the microbial assemblages. The comparison of microbial abundance, the proportion of gram-negative bacteria, gram-positive bacteria, and fungi was done using one-way ANOVA in SPSS 11.5.

The abundance of nematodes and the abundance of all other taxa were examined separately using repeated measures ANOVA in SPSS 11.5. Log transformation was done on the raw data in order to obtain a normal distribution. Repeated measures ANOVA was performed without Day 4 data, since an equal time interval is required for the analysis and equal time steps are present only between Day 0, Day 20, and Day 40. Abundances of arthropod and tardigrade feeding groups were also analyzed using repeated-measures ANOVA in SPSS.

The invertebrate assemblages were compared using ssh-MDS with PATN 3.03 (Belbin 1989). The Bray-Curtis method was used as it is the metric that best handles large numbers of zeros in the data set. Before analysis, all genera/families with only one observation were removed. Due to the lack of Day 40 data for the nematodes, the invertebrates were analyzed with all organisms excluding Day 40, and including Day 40 without the nematodes. The tests were performed on the raw data, log transformed data, and presence/absence data. ANOSIM was performed comparing the two treatments (Root and Leaf vs. Root Only). An ANOSIM was also performed comparing the two plant species to see whether there were species-specific invertebrate assemblages.

Monte-Carlo Attributes in Ordination (MCAO), which assigns each variable a vector based on correlation, was done to reveal which groups were important in creating the ordination.

RESULTS

Soil carbon

The 2004 results for soluble carbon showed that there was a significant increase in the amount of soluble carbon over time in both of the simulated herbivory treatments, 'Root and Leaf' herbivory and 'Root Only' herbivory (time $P<0.001$) (Fig. 3-3). While all the samples showed this increase in soluble carbon over time, presumably due to root herbivory having occurred in all pots, there was an added effect of leaf herbivory with the soluble carbon being higher in Root and Leaf pots than in Root Only (time*treatment $P=0.037$). In order to verify these results, the experiment was redone in 2005, with the additional separation of root and leaf herbivory to find out how each of these on their own affected the soil carbon.

In 2005 the results differed strikingly from those in 2004. When the results from the Day 2 coring were analyzed, there was a significant effect of treatment (Control, Root Only and Leaf Only) on the soluble carbon ($P=0.021$) with the Control having significantly higher soluble carbon in the soil than the Root Only treatment (Fig. 3-4). There was also a difference between the plant species ($P=0.033$; Fig. 3-4) in terms of soluble carbon, with primrose having significantly more soil carbon than fireweed. There was no interaction between treatment and plant species ($P=0.959$). In terms of the difference between treatments, the Control pots had more soluble carbon than Root Only

pots, while Leaf Only fell in between the two. Since the Root Only pots were cored twice, they were analyzed separately to see the differences between Day 0 and Day 2 in terms of soluble carbon. There was no difference between the two times (P=0.702; Fig. 3-5) and there was no difference between plant species (P=0.121). The *Pilea* experiment, testing the effects of root coring (Root Only) versus none (Control), yielded results that agree with those from the 2005 soluble carbon experiment; there was no treatment effect on the amount of soluble carbon in the soil (P=0.075; Fig. 3-6).

The raw data on the plant characters for the soluble carbon studies can be found in Appendix 3 (2004) and Appendix 4 (2005).

PLFA analysis of microbial assemblages

When the total microbial abundance of each leaf clipping treatment was compared, microbial biomass was lower in the Both treatment group (P=0.033; Table 3-1) as compared to both Old and Young treatments, which were similar. Control falls in between Both and the Old and Young group. When the percentages of the major groups of microbes were analyzed (gram positive bacteria, gram negative bacteria and fungi), there was no difference in any group between the treatments (Table 3-1). When analyzed in PATN, the ANOSIM revealed a significant difference at P<0.05 for the Control vs. Young, and for Both vs. Young (Table 3-2; Fig. 3-7). These were the only significant results obtained.

The raw data on the plant characters can be found in Appendix 5.

Invertebrate assemblages

Recall there is no Day 40 data for the nematodes due to the freezing accident. The results of the ANOVA on total nematode counts showed no effect of treatment (Root and Leaf vs. Root Only), with only time being significant ($P<0.001$). When nematode abundance was analyzed by trophic group, there was also no effect of treatment. For both species of plants and both treatments, the nematode numbers increase over the sampling period (Fig. 3-8 a & b).

For the ANOVA on total mite counts, there was a significant interaction for time*treatment, with more mites being present at the end of the experiment in the Root Only pots ($P=0.008$), while mite counts in the Root and Leaf pots did not change significantly over the course of the experiment (Fig. 3-9).

When the arthropod taxa (Acari and Collembola) were analyzed based on feeding type, there were significant time*treatment results for the microbivores and the carnivores ($P=0.011$ and $P=0.025$, respectively). Microbivores increased sharply between Day 0 and Day 20 in the Root Only pots, while this increase did not occur until between Day 20 and Day 40 in the Root and Leaf pots (Fig. 3-10). For the carnivores, in the Root Only pots the increase come between Day 20 and Day 40, while in the Root and Leaf pots there is a decrease over time (Fig. 3-11).

The ANOSIM performed on all invertebrates revealed that there was a significant difference between the two plant species in terms of invertebrate assemblages ($P<0.0001$; Fig. 3-12). The ordination did not reveal a pattern based on treatment (ANOSIM $P>0.1$; Table 3-3). ANOVA performed on abundance of invertebrates revealed a difference between the plant species. Arthropods were significantly more abundant in the fireweed

pots than in the primrose pots (P=0.001). For the nematodes, the same pattern held with more being present in the fireweed pots; however, this was significant only at Day 20 (P=0.005), with the Day 0 results being marginally non-significant (P=0.060).

In summary, the results for the soluble carbon differed between years: for 2004 there was a strong effect of simulated herbivory on the amount of soluble carbon in the soil while in 2005 neither trial revealed a significant effect of herbivory. The results for the PLFAs indicated there was a treatment effect on the soil microbial assemblages based on leaf age at clipping, although the results were not consistent across treatments. The herbivory treatments did not have an effect on nematode abundance in the pots, while mites showed a significant effect of time by treatment in the Root Only pots. When the arthropod taxa were analyzed based on feeding type, there was an effect of time by treatment for microbivores and carnivores, with an increase in carnivores the time step following an increase in microbivores. There was also a plant-species effect on the invertebrates with differences in abundance and assemblage between the fireweed and primrose pots.

DISCUSSION

Soluble carbon

The results for soluble carbon are mixed, as the results from the two years do not agree with each other. The 2004 experiment yielded a significant increase in the amount of soluble carbon in the soil due to herbivory. While it was evident that root herbivory alone had a great effect on the soluble carbon in the soil (see Fig. 3-3), there was an

added effect of leaf herbivory on the soluble carbon. However, these results were not supported by the experiment conducted in 2005 which showed the opposite effect of herbivory (leaf, root, or in combination) on the amount of soluble carbon in the soil. In contrast to 2004, the 2005 experiment had a significantly higher amount of soluble carbon present in the control group. The implication is that in 2005, herbivory decreased the amount of carbon released by roots. The reasons for this discrepancy are unknown, but may be related to some plant factor not measured or to climatic variation between the two years (e.g. solar energy reaching the greenhouse), as many factors can affect exudate quantity (Grayston *et al.* 1996). If the plants had experienced stress prior to the herbivory treatments, this may have an effect on the results, as an increase in exudation is a short term response to stress while a long term effect of prolonged stress is to reduce carbon allocation to the roots and increase allocation to the shoots (Bardgett *et al.* 1998).

Some of the previous studies on the effects of herbivory on soil carbon have sampled repeatedly over time to show how the carbon inputs into the system increase in response to herbivory (Holland *et al.* 1996; Grayston *et al.* 1996; Coleman *et al.* 2002). Using this method they were able to monitor the changes in the quantity of exudates that were released into the soil. With the soluble carbon measure used in my study, we simply get a 'snapshot' of the soil carbon and may miss the increase or decreases that occur if the sampling time does not coincide with the carbon flux.

Effects of leaf age at clipping on soil microbes

The results of the PLFA analysis were also puzzling. While there were no differences in microbial assemblages between the Old and the Young leaf pots, there was

a significant difference between these treatments and the Both treatment, which was a combination of the previous two. This was also evident in the abundance of microbes between the treatments: the Both treatment had the lowest while Young had the highest. The implications are that clipping either young or old leaves increases the biomass of microbes in the soil while clipping a mixture of leaf ages causes a decrease in microbial abundance. In the Control treatment, there was a greater microbial abundance than for Both, with lower abundance when compared to the Old and Young treatments.

The effect of leaf clipping on the Old and Young treatments in comparison to the Control makes sense in terms of plants shunting carbohydrates to the roots in response to herbivory (Wardle 2002). The increased microbial biomass in these two treatments in comparison to the control supports the bottom-up control as well: the clipping may have increased the amount of exudates being released into the soil and therefore the microbes in the rhizosphere had a greater amount of nutrients to consume. What is puzzling is why the Both treatment, in which both old and young leaves were clipped, showed a decrease in the microbial biomass in comparison to the control. If these plants had shown the same response to herbivory that the other two treatments had, we would expect an increase in the microbial abundance for the Both treatment as well. Fireweed is known to have defensive compounds that are found in the roots (Rauha *et al.* 2000; Battinelli *et al.* 2001) and that might negatively affect microbes, yet why they would only be a factor when a combination of leaf ages were clipped is unclear.

Previous studies on plant age and herbivory have focused on the effect of these two factors on defensive compounds in leaves (Bower and Stamp 1993; Lambdon *et al.* 2003). In a study on *Plantago*, Bowers and Stamp (1993) found that the plant reduces the

amount of defensive chemicals in leaves as it ages, unless there is herbivory. In that study, new and intermediately aged leaves were herbivorized. Lambdon *et al.* (2003) found that younger *Brassica* leaves had higher amount of defensive compounds than older leaves. Neither study mentioned the potential effects on soil biota. With the recent increased interest in understanding the connectedness of the above-ground and below-ground systems, this aspect provides an intriguing area of research and warrants further investigation.

Invertebrate assemblages

The lack of Day 40 data for the nematodes due to freezing makes it hard to fully understand what is happening among the invertebrates. In both of the treatments (Root Only, Root and Leaf), the nematodes increased over time in all of the pots (Fig. 3-3). This increase may be due to an increase in soil carbon in response to simulated herbivory, or it may simply be due to the nematodes increasing in number over the growing season in response to changes in the soil temperature and moisture (Bardgett *et al.* 1997; and see Chapter 2 for seasonal increases in invertebrates). Previous studies on the effects of defoliation on nematode numbers have shown herbivory to increase the number of microbivorous nematodes in the soil system (Bardgett *et al.* 1997; Wardle *et al.* 2004). Zelenev *et al.* (2004) have shown that nutrient inputs into the soil cause the bacteria-eating nematodes to increase in a wave-like fashion, highlighting the need to sample repeatedly in the system to see the whole picture.

I had expected that the total mite numbers in the herbivorized pots would increase throughout the experiment as a flow-on effect of carbon additions caused by increased

root exudation in response to herbivory (Grayston *et al.* 1996; Holland *et al.* 1996) increasing the number of prey items such as nematodes. Other studies have shown defoliation to cause an increase in the abundance of nematodes (Ingham and Delting 1984; Seastedt *et al.* 1988; Bardgett *et al.* 1997; Mikola *et al.* 2005). I did not find the expected bottom-up pattern in mite numbers: mite abundances were relatively constant throughout the experiment in the Root and Leaf treatment while they increased in the Root Only pots. One potential explanation for this difference is that along with sugars being exuded by the roots, there may also have been a release of plant secondary compounds in response to the leaf herbivory, and that these compounds may have indirectly or directly inhibited population growth of soil invertebrates. Both of the plant species examined in this current study are known to release compounds that affect yeast, fungi, and bacteria (Shukla *et al.* 1999; Rauha *et al.* 2000; Battinelli *et al.* 2001). These compounds, depending on their toxicity, could suppress microbial populations and so would affect organisms that require microbes as a food source. This would in turn affect those in higher trophic levels, which may explain why mite populations in the Root Only pots increased while remaining the same in the Root and Leaf pots. However, this becomes problematic when the nematodes are considered, since they do not show a similar pattern. This could simply be caused by the lack of Day 40 data for the nematodes: we do not know what their abundances might have been at the end of the experiment, and they could have shown the same results as the mites. It is also possible that some root exudates can have toxic effects on arthropods alone. Plant secondary compounds can negatively affect root-feeding insect larvae and the nematodes that feed

on these larvae (van der Putten 2003). How these compounds might affect higher trophic levels are yet to be studied (van der Putten 2003).

Bottom-up effect of herbivory

The effect of herbivory on the soil invertebrate trophic groups does not support the original hypothesis that the herbivory would cause a sequential increase in the amount of microbivores followed by carnivores. If herbivory invariably causes this effect, it would have been clear in both treatments. Also, the lack of an adequate control (no herbivory) makes comparisons and conclusions difficult at best. With these caveats in mind when examining the Root Only pots, there is some evidence of a bottom-up effect, with the microbivorous arthropods increasing first at Day 20, then the carnivores increasing at the next time step, Day 40 (Fig. 3-10). While the Root and Leaf pots did not show this, even with a greater amount of herbivory, it may have been that the sampling time was not long enough. If the exudates did indeed contain secondary compounds produced in response to the leaf herbivory that had an inhibitory effect on the soil organisms, the bottom-up effect would not be as clearly visible. Total mite abundance in the Root and Leaf pots appears static, but when the trophic groups are analyzed, we see that the microbivores showed an increase in the final sampling time, Day 40 (Fig. 3-10). Had the sampling continued, then we may have seen the increase in the carnivores at the next time step. One important thing to note is that 'carnivores' may feed on nematodes, not just on other arthropods. And also, there is no reason why they would limit their predation on arthropods to microbivores. This highlights the need to know the soil food web in detail in order to begin to make predictions and explanations on this system.

Conclusion

Based on these results, it would appear that simulated herbivory does not provide evidence for a bottom-up control in the arthropod trophic groups. Results for the nematodes could be adequately used, mostly due to the lack of data for the final sampling period. While it may seem that in the Root Only pots there was weak evidence for bottom-up control, the lack of a control and the lack of a similar response in the Root and Leaf pots make this conclusion shaky. In the case of both simulated root and leaf herbivory, there may be something else occurring in this system causing a suppression of the arthropod population growth and a delay in the microbivore increase. This could be due to secondary compounds being released into the soil in response to the leaf herbivory, or to some other factor not examined in this study. I was unable to consistently determine the influence of herbivory on the amount of soil carbon as evidence for an increase differed between years. This may be due to the sampling method used, as I tested soluble soil carbon in a one-shot sampling period whereas repeated sampling gives data over a longer time period, increasing the chances of seeing changes in the system. One way to solve this problem would be to take soluble carbon samples over time in order to see changes that may be occurring in response to herbivory. The effect of leaf age at clipping also yielded inconsistent results, as the clipping of either old or young leaves caused an increase in microbial abundance whereas when a combination of leaf ages were clipped there was a decrease in abundance. It is intriguing that there was so much variation in effects of simulated herbivory on assemblages of invertebrates and microbes. Clearly this area deserves further examination if we are to understand the connections between above- and below-ground biota.

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TABLES AND FIGURES

Table 3-1: Mean microbial biomass (nmol / g dry soil) in the rhizosphere of fireweed pots tested for the effect of leaf age at clipping on the microbial assemblages. The % breakdown for gram positive, gram negative and fungal contributions are also shown. Treatments involved 60% removal of old, young, and combination of both. Control had no leaves clipped. The % PLFA's do not add up to 100% as only the PLFAs most representative of each group are used. Groups were created using Tukey's test after a one-way ANOVA in SPSS, based on $P<0.05$.

	Control	Both	Old	Young
Total abundance	142.18^{ab}	123.98^{a}	157.40^{ab}	170.72^{b}
% Gram negative	17.6^{a}	17.7^{a}	18.5^{a}	18.7^{a}
% Gram positive	26.1^{a}	27.4^{a}	26.2^{a}	26.2^{a}
% Fungal	19.6^{a}	18.9^{a}	18.6^{a}	18.3^{a}

Table 3-2: ANOSIM similarity between treatments of leaf age at clipping in terms of the microbial assemblages for fireweed grown in the greenhouse during the summer of 2005. Results were obtained using individual PLFAs as nmol / g dry soil. The p-value is shown.

	Control	Old	Young
Old	0.1021		
Young	0.0070	0.6917	
Both	0.4094	0.0721	0.0050

Table 3-3 – ANOSIM results for comparisons of invertebrate assemblages between Root Only and Root and Leaf pots. The data set used is indicated, either excluding all nematode data due to lack of Day 40 data, or excluding Day 40 data to see the effects of nematodes and arthropods together. The data transformation is also indicated, raw, log transformed, or only presence absence data.

Plant species	Data set	Transformation	ANOSIM p-value
Fireweed			
	Excluding nematodes	Raw	0.3013
		Logarithmic	0.1862
		Presence/absence	0.1181
	Excluding Day 40	Raw	0.1161
		Logarithmic	0.1261
		Presence/absence	0.3373
Primrose			
	Excluding nematodes	Raw	0.3173
		Logarithmic	0.3413
		Presence/absence	0.4304
	Excluding Day 40	Raw	0.4004
		Logarithmic	0.2553
		Presence/absence	0.3534

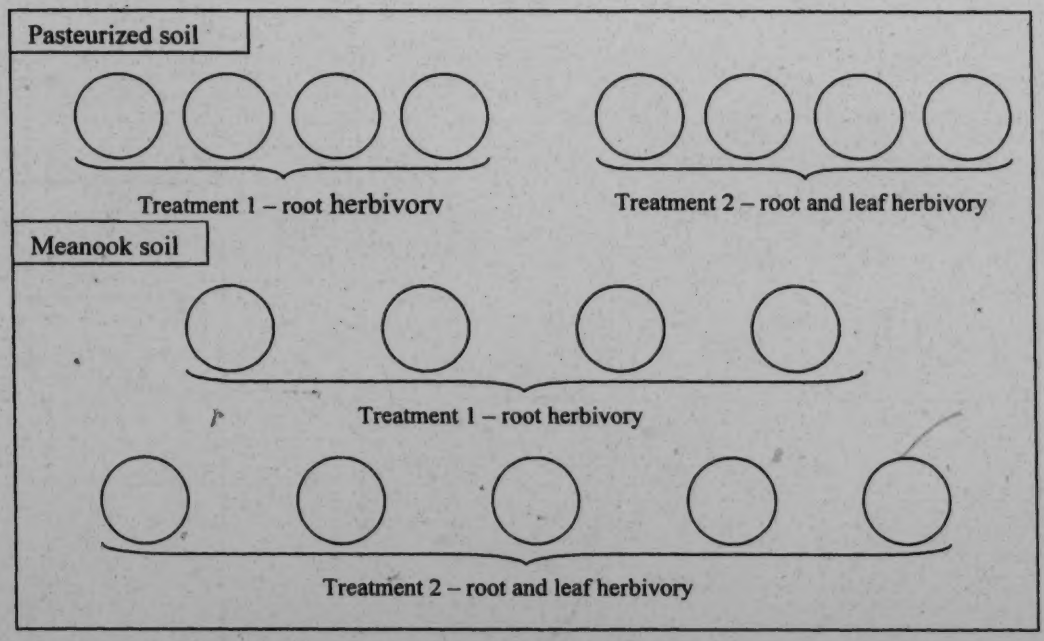

Pasteurized soil
Treatment 1 – root herbivory
Treatment 2 – root and leaf herbivory
Meanook soil
Treatment 1 – root herbivory
Treatment 2 – root and leaf herbivory

Figure 3-1 – Sampling design for greenhouse experiment, showing how many pots per soil type (pasteurized vs. Meanook) and how many per treatment (treatment 1 vs 2). The design shown is for only one plant species and is identical for both species.

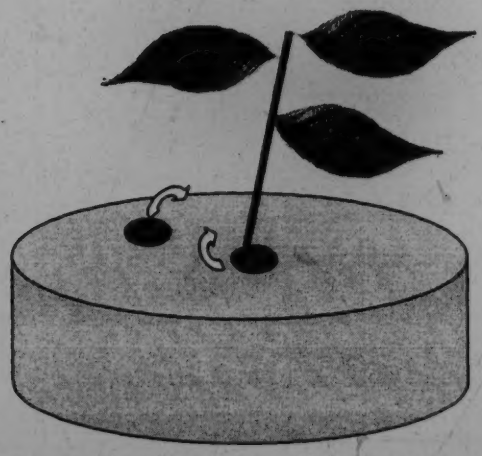


Figure 3-2 – Core sampling locations within the greenhouse pots. Arrows indicate the direction core location was moved for subsequent sampling. One was near the plant stem and other one further out. Each core was 2.5 cm in diameter and 5 cm deep.

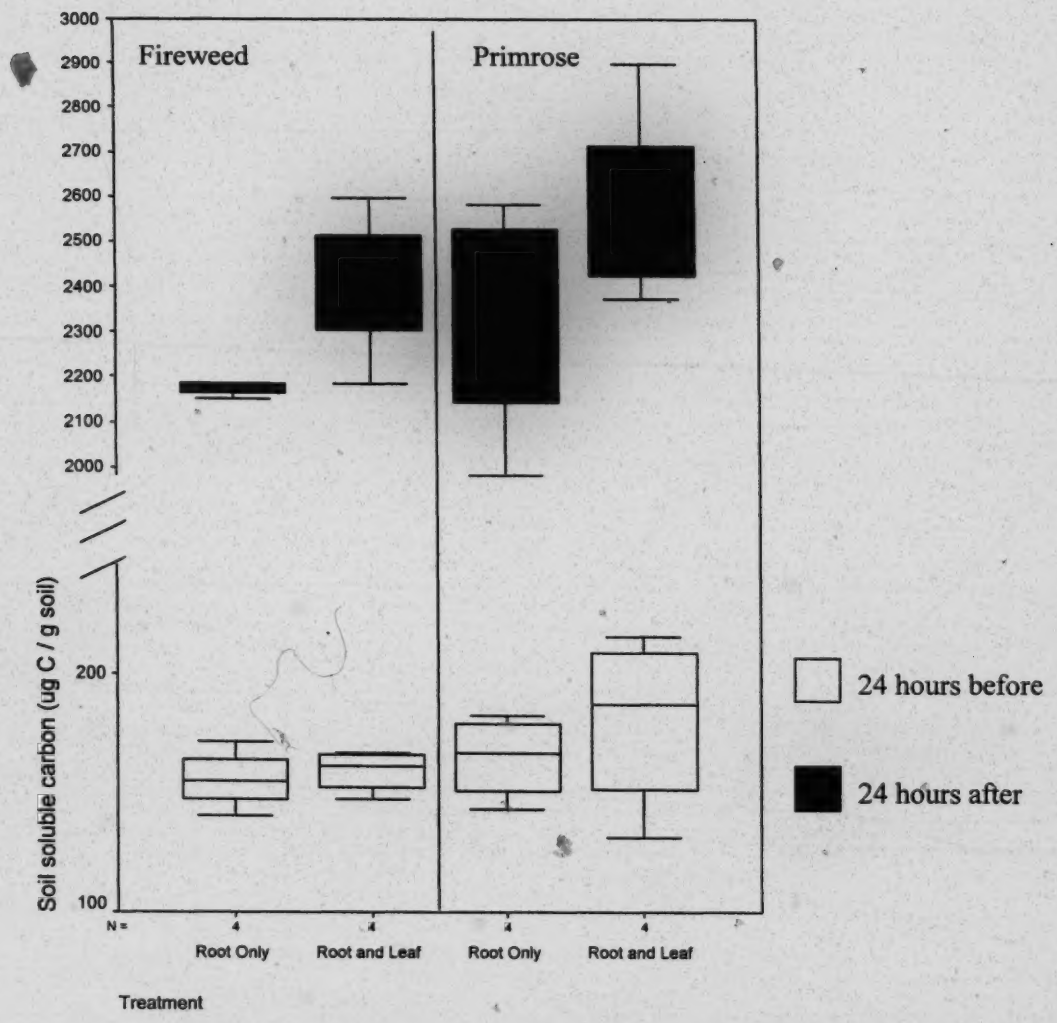


Figure 3-3 – 2004 soluble carbon distribution extracted from greenhouse pots 24 hours before (open) and 24 hours after (grey) defoliation separated by treatment and plant species. Note the change in scale after the axis break.

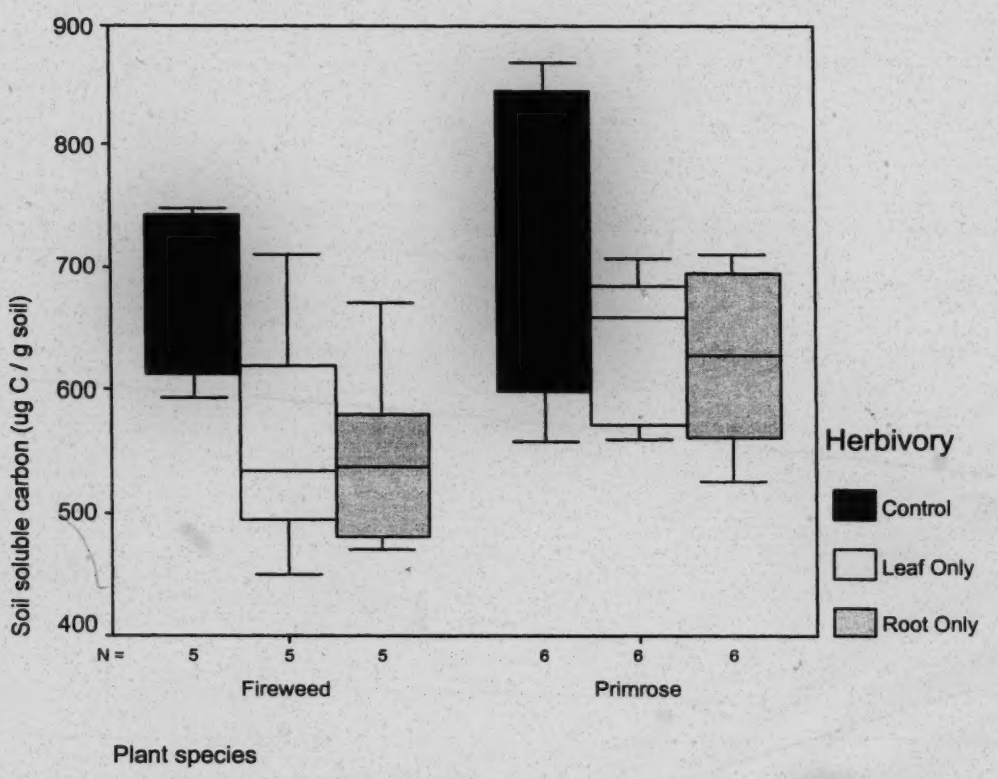


Figure 3-4: 2005 soluble carbon results separated by treatment and plant species taken on Day 2. Root Only had 2 cores taken, one at Day 0 (root herbivory) and Day 2 (sampling). Leaf Only had 60% of leaves clipped on Day 1 and were cored Day 2. The Control had one core taken on Day 2 only. There is a significant difference between the Root and Control pots (P=0.021).

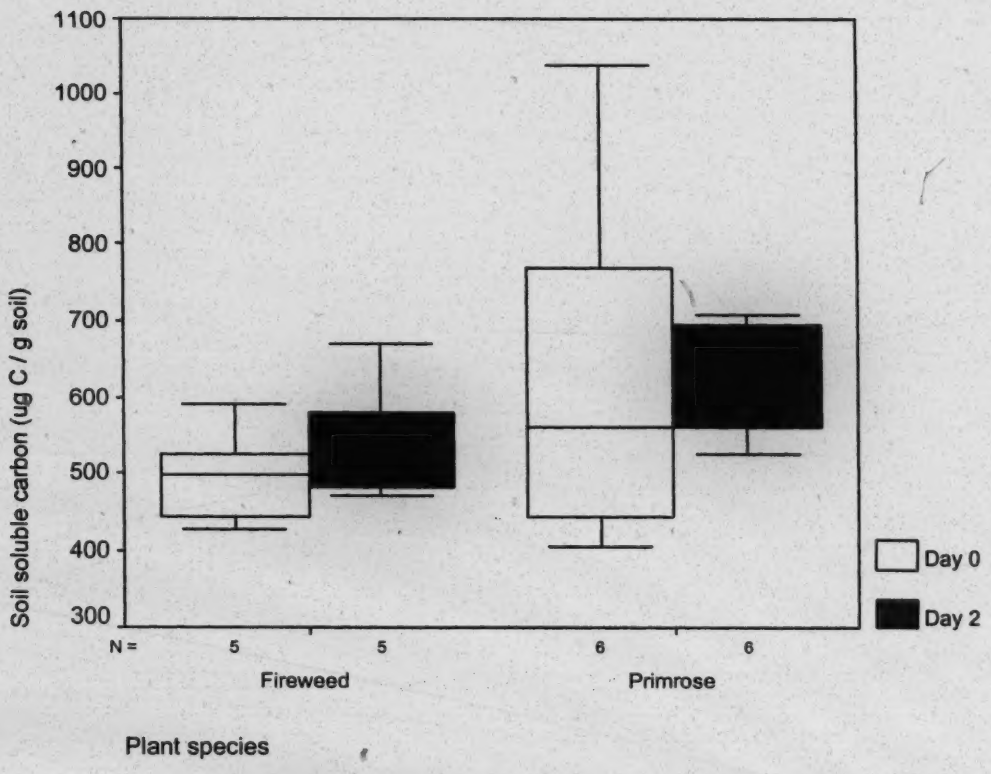


Figure 3-5: 2005 soluble carbon for the Root Only experimental pots separated by plant species. Day 0 (initial root herbivory) is solid grey and Day 2 (sampling) is checkered. There was no difference between the two times in terms of soluble carbon (P=0.702).

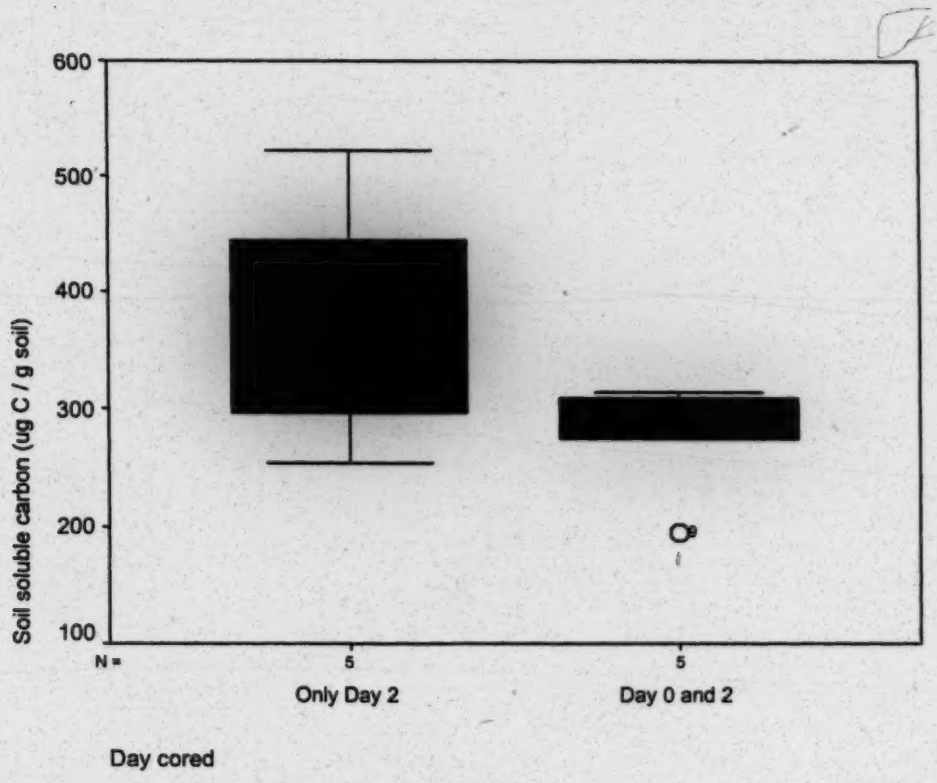


Figure 3-6: 2005 soluble carbon results from Day 2 coring for *Pilea*. The control was only cored on Day 2 while the Root Only was cored on Day 0 and again on Day 2. No significant difference ($P>0.05$).

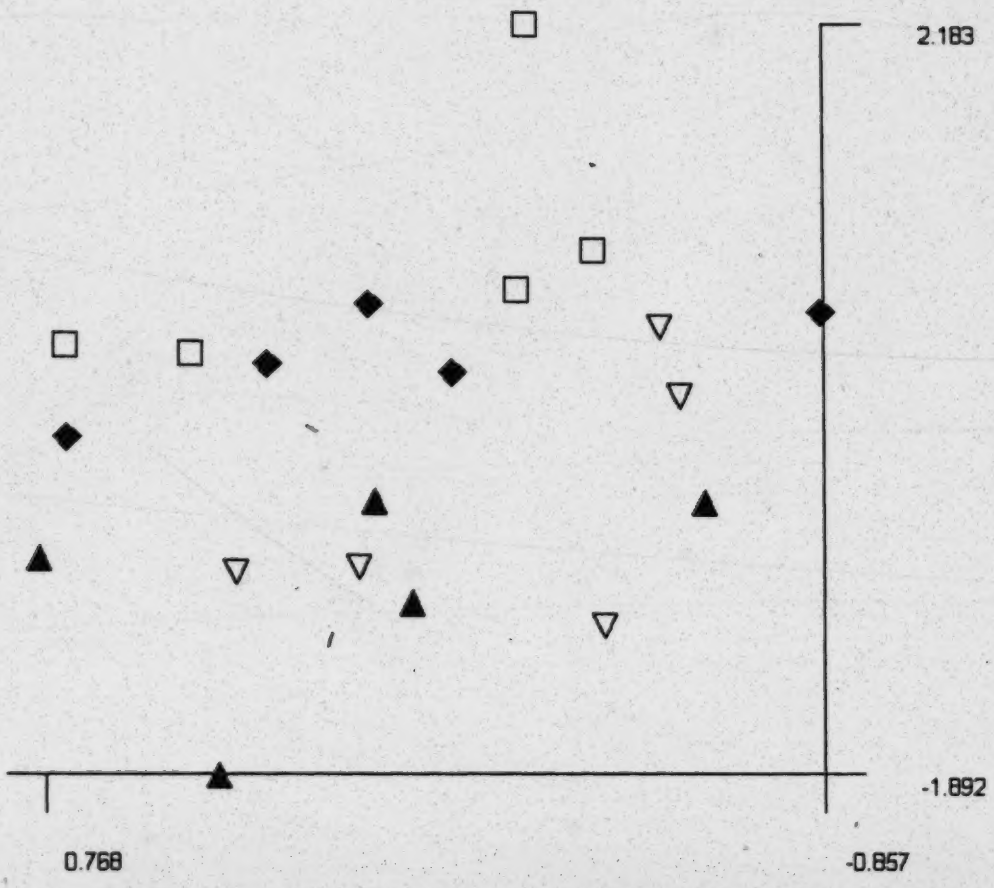
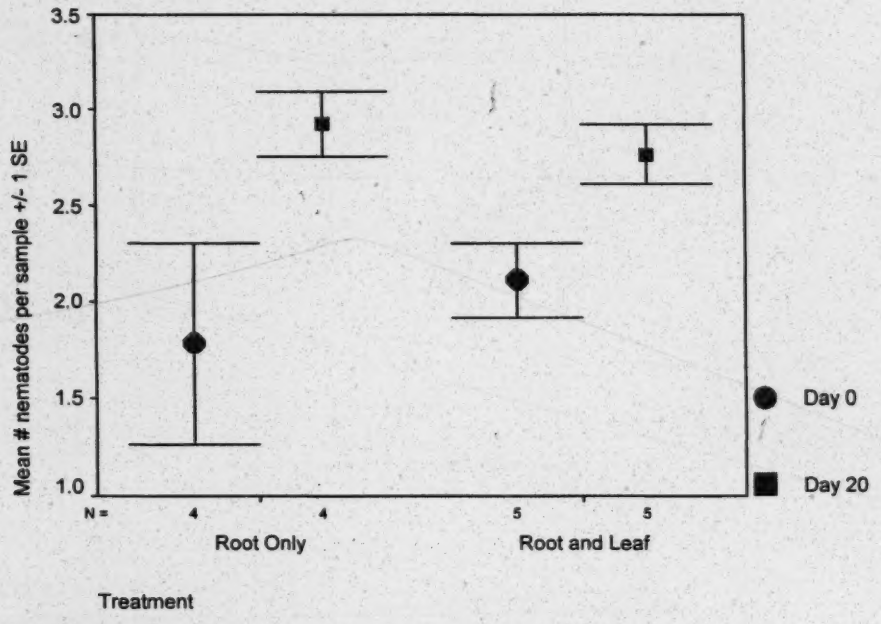


Figure 3-7: PATN 2-D ordination of leaf age treatments using PLFA analysis. The ordination stress is 0.1421. Closed diamonds indicate the Controls; open triangles indicate Old; Closed triangles indicate Young, and open squares are Both.

a) Primrose



b) Fireweed

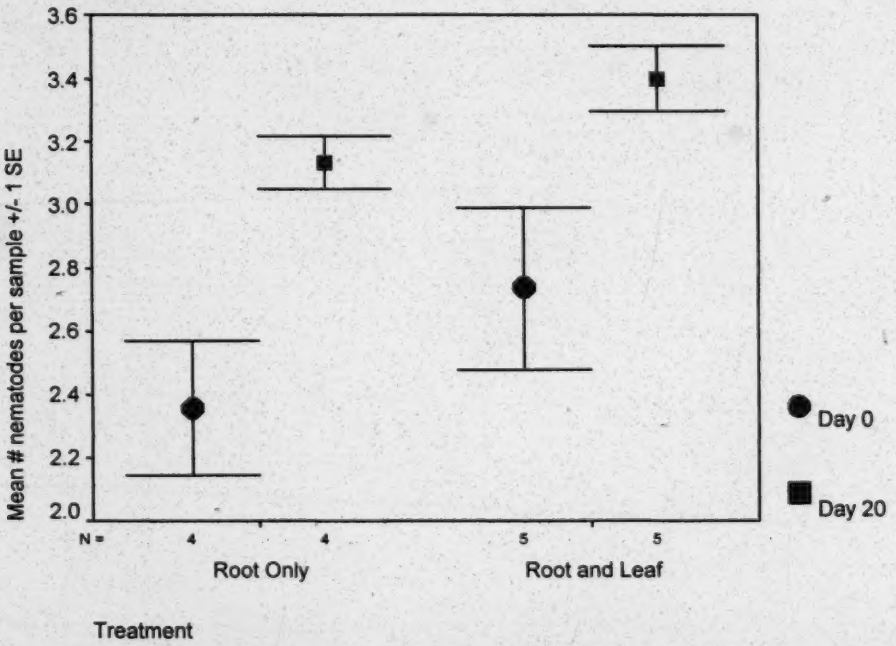


Figure 3-8 – Nematode abundance in the greenhouse, log transformed, for a) primrose pots, and b) fireweed pots. The circle indicates Day 0, before treatment was imposed, and the square indicates 20 Days after defoliation. Note the scale differences.

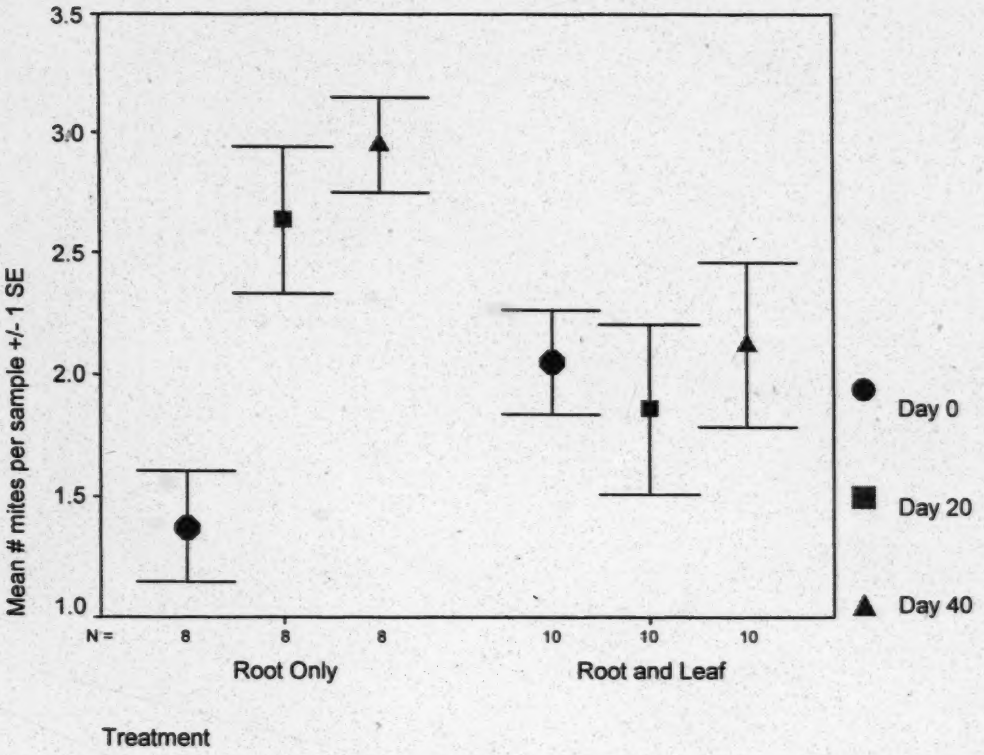


Figure 3-9 – Mite abundance, log transformed, with both plant species combined. The plant species were combined as there was no difference between the species over time. The circle indicates Day 0, before treatment was imposed, the square indicates 20 days after defoliation, and the triangle indicates 40 days following treatment.

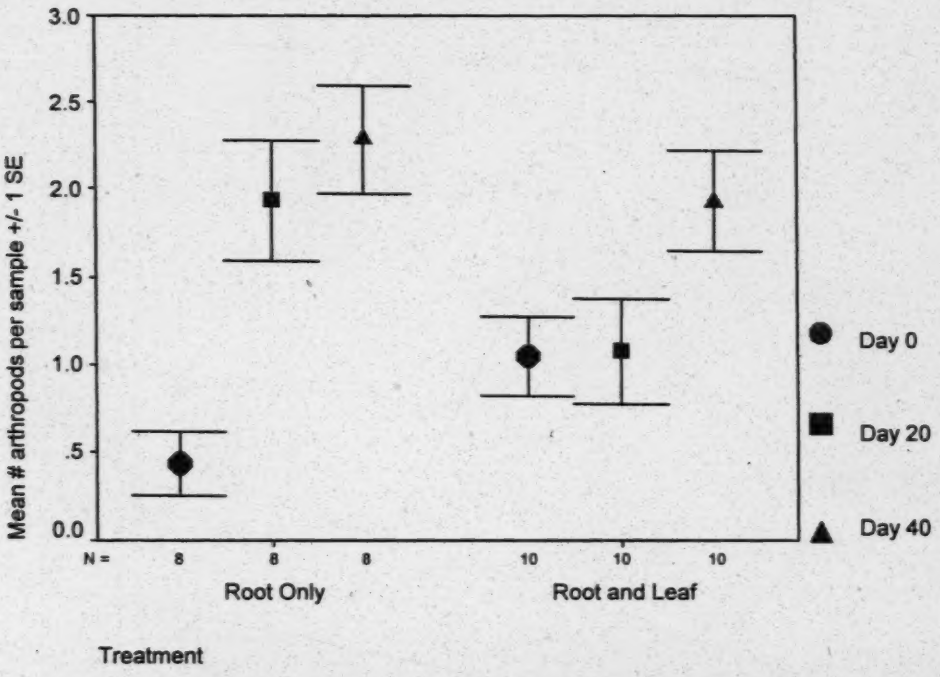


Figure 3-10 – Microbivorous arthropod abundance in greenhouse, log transformed, with both plant species combined. The plant species were combined as there was no difference between the species over time. The circle indicates Day 0, before treatment was imposed, the square indicates 20 days after defoliation, and the triangle indicates 40 days following treatment.

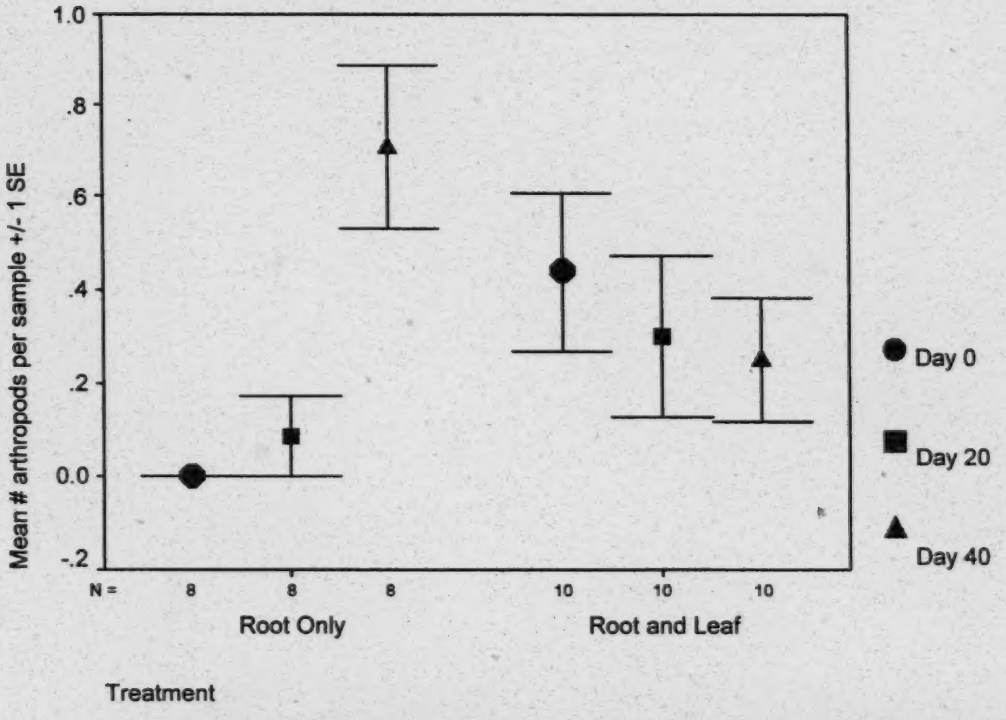


Figure 3-11 – Carnivorous arthropod abundance in greenhouse, log transformed, with both plant species combined. The plant species were combined as there was no difference between the species over time. The circle indicates Day 0, before treatment was imposed, the square indicates 20 days after defoliation, and the triangle indicates 40 days following treatment.

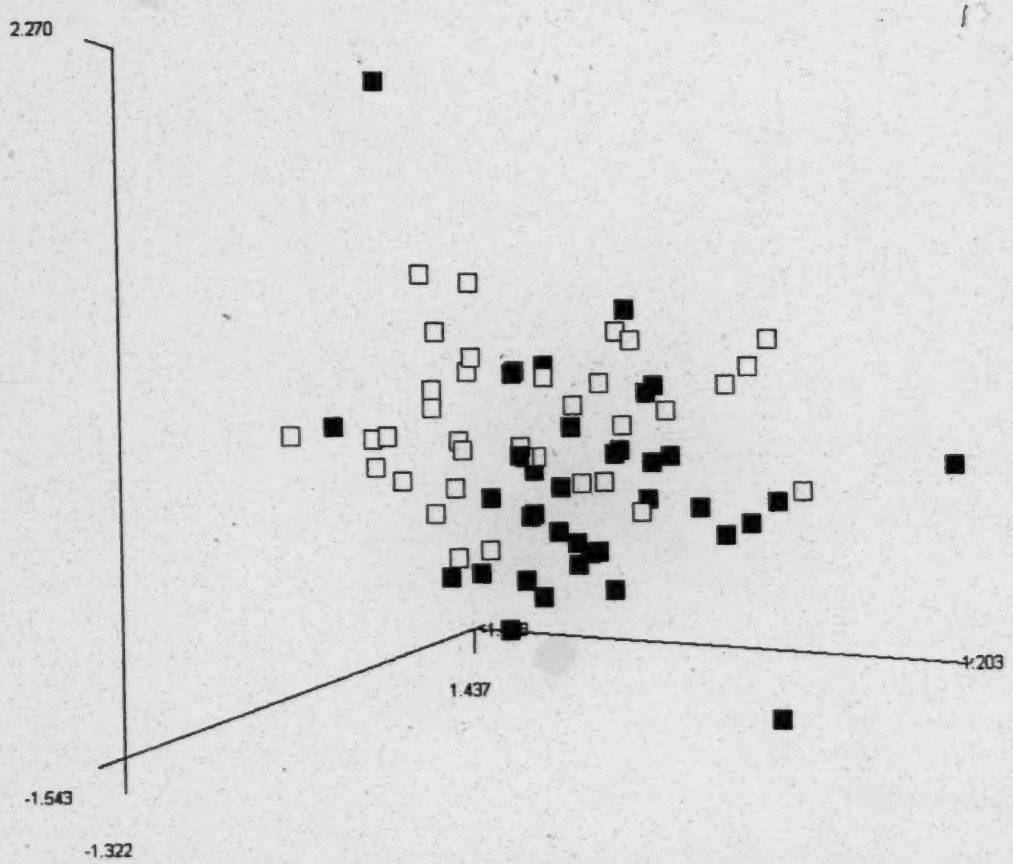


Figure 3-12 – Ordination plot for all 2004 invertebrate samples (excluding nematodes) separated by plant species. The open squares indicate the fireweed samples while the black squares indicate the primrose samples. The ordination stress for 3D was 0.2129, and the ANOSIM between plant species was $P<0.0001$.

SUMMARY AND CONCLUSION

I investigated the effects of plant species and herbivory on soil organisms associated with the rhizosphere of boreal plants. Plants are the connectors between the above- and below-ground ecosystems and so they have a direct effect on the organisms near their roots (Wardle 2002). Plants release nutrients in the form of root exudates into the soil system, which are then in turn consumed by microbes (Grayston *et al.* 1996). Microbivores and predators then also benefit from these plant resources through their food source, the microbes. Plant species are known to have their own unique exudates and this has been shown to yield unique microbial assemblages within the rhizosphere of plants (Grayston *et al.* 1996; Wardle 2002). Whether this translates into microbivores and carnivores also being plant specific was one of the questions being asked in Chapter 1. Herbivory can also have an effect on the root exudates, as herbivory (above- or below-ground) causes a short term increase in the amount of carbon being exuded into the soil system (Holland *et al.* 1996; Grayston *et al.* 1996; Yeates *et al.* 1998; Grayston *et al.* 2001). This can then cause an increase in the microbes, which are primarily controlled in a bottom-up fashion through nutrient availability (Bardgett *et al.* 1998; Yeates *et al.* 1998; Denton *et al.* 1999). In Chapter 2, effects of both root and leaf herbivory were tested.

In Chapter 1, I compared the soil invertebrate assemblages of four boreal herbaceous plants (anemone, fireweed, northern bedstraw, and common vetch), as well as the functional assemblages of the bacteria, to determine if they are specific to these plant species. I also wanted to examine how these assemblages change over the summer

season. This was done by sampling these four plant species at four separate times over the growing season in two sampling plots set up at the Meanook Biological Research Station. For bacteria, there were strong differences between the two plots, which was correlated with light intensity and coarse woody debris, with higher values of both associated with a higher carbon usage pattern. Using multivariate statistics, I determined that carbon usage pattern of the bacteria was specific to particular plant species. However, this did not translate into plant specificity for the soil invertebrates. There was no effect of plant species on invertebrate assemblages, although there were clear seasonal patterns with a greater abundance and diversity of organisms found later in the growing season.

To further test the effects of exudates on soil assemblages, I conducted a greenhouse experiment to determine how below-ground herbivory, alone and in conjunction with above-ground herbivory, affects the amount of soluble carbon in the soil, and whether this flows on to affect invertebrates. I expected that soluble carbon would increase in response to herbivory and that soil invertebrate numbers would increase due to this nutrient input. The soluble carbon results from 2004 and 2005 were contradictory, with 2004 results showing an increase in response to herbivory while those from 2005 did not. The reason for this discrepancy is unknown; it may relate to differences in the plants themselves between years, or to the time-window in which the core was taken, as there may have been seasonal differences that caused a change in the timing of carbon release into the soil. The conditions in the pot may have been more favourable to microbial growth in 2005, therefore any carbon released may have been taken in by microbes before the sampling took place. Further tests, such as repeated

sampling to track changes in soil carbon or stable isotope analysis, would help clarify what is happening to the root exudates in response to herbivory in these plants.

The soil invertebrate response to simulated herbivory was tested only in 2004. Pots with only root herbivory showed an increase in microbivorous arthropods followed by an increase in carnivores. For the arthropods in these pots, the hypothesis of bottom-up control seems to be supported, however the lack of adequate control (no herbivory) and the lack of a response in the other herbivory treatment cast doubt on this conclusion. For the pots with both above- and below-ground herbivory, there was only an increase in the microbivorous arthropods at the last sampling time. As there appeared to be some un-measured factor causing a suppression of invertebrate abundance in the Root and Leaf herbivory pots, the increase in abundance of carnivores may have been delayed. It is possible that had the sampling continued for another time step that an increase in the carnivores would have been observed in response to the increase in microbivores.

I also examined the effect of leaf age at time of clipping, with above ground herbivory consisting of removal of young leaves only, old leaves only, or a combination of young and old leaves. The effect of this on the soil microbial assemblage was analyzed. The young-only and old-only treatments showed similar results in terms of changes in microbe assemblages, whereas the young + old treatment behaved more like the non-herbivorized control pots, and indeed ended up with the lowest microbial biomass. This was puzzling, and no adequate explanation was found.

The results of the experiments above show that plants have a profound effect on the soil system. This is understandable as plants are the connectors between these two systems, shunting photosynthetic products from the above-ground system to the below-

ground system, where they can enter the soil as root exudates. These exudates affect the soil system: through stimulating microbial growth (Wardle 2002), and indirectly providing a food source for other soil organisms; and also through defensive compounds that may be released along with these sugars (Grayston *et al.* 1996; Walker *et al.* 2003). Any action that changes the exudation pattern of the plant will have repercussions in the soil food web. Future research should be aimed towards examining more closely the root exudates released by plants. This will allow us to better understand this system and how best to predict and explain changes in assemblages of soil biota across space and time.

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Appendix 1: Field data collected during the 2004 summer season from the Meanook Biological Research Station forest. The Plot (1 vs. 2), the sampling date and the plant species are listed for each sample. The light intensity (lm/m^2), soil temperature (°C), and soil moisture (available water to a depth of 10cm) were taken at each plant directly before cores were taken.

Sample	Plot	Date	Species	Light	Temperature	Moisture
A01	1	10-Jun-04	*Anemone canadensis*	5338.9	11.8	0.27
A02	1	10-Jun-04	*Anemone canadensis*	3993.4	10.2	0.80
F01	1	10-Jun-04	*Epilobium angustifolium*	5737.2	10.9	0.53
F02	1	10-Jun-04	*Epilobium angustifolium*	5091.3	10.1	0.53
G01	1	10-Jun-04	*Galium boreale*	4111.8	11.6	1.04
G02	1	10-Jun-04	*Galium boreale*	4908.3	10.5	1.04
V01	1	10-Jun-04	*Vicia americana*	6221.5	9.7	0.53
V02	1	10-Jun-04	*Vicia americana*	6684.4	11.3	0.53
A03	2	10-Jun-04	*Anemone canadensis*	10344.1	10.2	0.80
A04	2	10-Jun-04	*Anemone canadensis*	8191.3	9.6	0.53
F03	2	10-Jun-04	*Epilobium angustifolium*	9052.4	10	0.53
F04	2	10-Jun-04	*Epilobium angustifolium*	7222.6	9.7	1.04
G03	2	10-Jun-04	*Galium boreale*	8901.8	10	0.27
G04	2	10-Jun-04	*Galium boreale*	9601.4	10.4	0.53
V03	2	10-Jun-04	*Vicia americana*	7319.5	11.3	1.04
V04	2	10-Jun-04	*Vicia americana*	9741.3	9.5	0.53
A05	1	14-Jul-04	*Anemone canadensis*	19375.0	16.6	1.04
A06	1	14-Jul-04	*Anemone canadensis*	6253.8	14.7	1.04
F05	1	14-Jul-04	*Epilobium angustifolium*	10182.7	15.8	1.04
F06	1	14-Jul-04	*Epilobium angustifolium*	11625.0	15.8	1.04
G05	1	14-Jul-04	*Galium boreale*	13670.2	15	0.53
G06	1	14-Jul-04	*Galium boreale*	32076.5	14.4	1.04
V05	1	14-Jul-04	*Vicia americana*	42302.2	15.7	1.04
V06	1	14-Jul-04	*Vicia americana*	11517.4	14.3	1.33
A07	2	14-Jul-04	*Anemone canadensis*	3713.5	16.9	0.53

A08	2	14-Jul-04	*Anemone canadensis*	42194.5	14.7	1.04
F07	2	14-Jul-04	*Epilobium angustifolium*	17114.6	15.4	1.04
F08	2	14-Jul-04	*Epilobium angustifolium*	12809.1	15.2	0.80
G07	2	14-Jul-04	*Galium boreale*	77607.8	16.1	0.53
G08	2	14-Jul-04	*Galium boreale*	62107.8	15.6	0.80
V07	2	14-Jul-04	*Vicia americana*	36274.4	15.9	0.80
V08	2	14-Jul-04	*Vicia americana*	86864.8	15.5	0.80
A09	1	19-Aug-04	*Anemone canadensis*	8019.1	13.7	0.53
A10	1	19-Aug-04	*Anemone canadensis*	9181.6	14.1	0.53
F09	1	19-Aug-04	*Epilobium angustifolium*	11851.1	13.9	0.80
F10	1	19-Aug-04	*Epilobium angustifolium*	31538.3	14.8	0.53
G09	1	19-Aug-04	*Galium boreale*	6221.5	14	0.53
G10	1	19-Aug-04	*Galium boreale*	6867.4	13.7	0.53
V09	1	19-Aug-04	*Vicia americana*	9579.9	13.6	0.53
V10	1	19-Aug-04	*Vicia americana*	8643.4	13.8	0.53
A11	2	19-Aug-04	*Anemone canadensis*	78899.5	14	0.53
A12	2	19-Aug-04	*Anemone canadensis*	6350.7	13.7	0.80
F11	2	19-Aug-04	*Epilobium angustifolium*	12367.7	13.6	0.53
F12	2	19-Aug-04	*Epilobium angustifolium*	14014.6	14	0.53
G11	2	19-Aug-04	*Galium boreale*	15769.1	14	1.04
G12	2	19-Aug-04	*Galium boreale*	76316.1	13.3	0.80
V11	2	19-Aug-04	*Vicia americana*	22927.1	13.6	0.53
V12	2	19-Aug-04	*Vicia americana*	18836.8	14	0.53
A13	1	21-Sep-04	*Anemone canadensis*	5941.7	7.7	0.53
A14	1	21-Sep-04	*Anemone canadensis*	5478.83	8.1	0.80
F13	1	21-Sep-04	*Epilobium angustifolium*	10365.7	7.7	0.80
F14	1	21-Sep-04	*Epilobium angustifolium*	9816.7	8	0.53
G13	1	21-Sep-04	*Galium boreale*	8299.0	7.6	1.04

G14	1	21-Sep-04	*Galium boreale*	9515.3	7.9	1.04
V13	1	21-Sep-04	*Vicia americana*	9644.5	8.1	1.04
V14	1	21-Sep-04	*Vicia americana*	7319.5	8.4	0.80
A15	2	21-Sep-04	*Anemone canadensis*	2906.3	8.1	1.04
A16	2	21-Sep-04	*Anemone canadensis*	3864.2	8	0.80
F15	2	21-Sep-04	*Epilobium angustifolium*	4133.3	8.3	0.53
F16	2	21-Sep-04	*Epilobium angustifolium*	4294.8	8.2	0.80
G15	2	21-Sep-04	*Galium boreale*	2163.5	8.2	0.80
G16	2	21-Sep-04	*Galium boreale*	11248.3	7.5	0.53
V15	2	21-Sep-04	*Vicia americana*	3842.7	8.3	0.53
V16	2	21-Sep-04	*Vicia americana*	6275.4	8.4	0.80

Appendix 2: Taxonomic list of organisms collected from Meanook and the greenhouse study using Meanook soil in 2004. + indicates found, - indicates not found

Order	Sub-order	Super-cohort	Cohort	Family	Genus	Greenhouse	Meanook
Sarcoptiformes	Endeostigmata		Alicorhagiina	Alicorhagiidae	*Alicorhagia*	+	+
				Nanorchestidae	*Nanorchestes*	-	+
	Oribatida	Enarthronotides		Brachychthoniidae	*Sellnickochthonius*	+	+
					Liochthonius	+	+
				Atopochthoniodea	*Atopochthonius*	-	+
		Parhyposomatides		Gehypohthoniidae	*Gehypochthonius*	-	+
		Mixonomatides		Oribotritiidae	*Protoribotria*	+	+
		Desmonoatides	Nothrina	Camisiidae	*Platynothrus*	+	-
				Trhypochthoniidae	*Trhypochthonius*	+	-
			Brachypylina	Plateremaeidae	*Allodamaeus*	-	+
				Cepheidae	*Sphodrocepheus*	-	+
				Peloppiidae	*Ceratoppia*	-	+
				Carabodidae	*Carabodes*	-	+
				Tectocepheidae	*Tectocepheus*	+	+
				Oppiidae	*Oppiella*	+	+
				Quadroppiidae	*Quadroppia*	+	+
				Suctobelbidae	*Suctobelbella*	+	+
					Rhynchobelba	-	+
				Oribatulidae	*Zygoribatula*	+	-
				Haplozetidae	*Peloribates*	-	+
				Scheloribatidae	*Scheloribates*	+	+
				Parakalummidae	*Neoribates*	-	+
				Ceratozetidae	*Ceratozetes*	+	+
					Fuscozetes	-	+
				Galumnatidae	*Galumna*	-	+
					Acrogaumna	-	+
					Pergalumna	+	+

					Pilogalumna	-	+
			Astigmatina			-	+
Mesostigmata					Immature	+	+
	Monogynaspida		Uropodina	Uropodidae		-	+
			Gamasina	Zerconidae	Immature	+	+
					Zercon	-	+
					Hypozercon	-	+
					Monozercon	-	+
					Microzercon	-	+
					Comsozercon	-	+
					Parazercon	-	+
				Ascidae	*Asca*	+	+
					Arctoseius	+	+
				Phytoseiidae		+	-
				Digamasellidae	*Dendrolaelaps*	-	+
				Gamasellidae	*Gamasellus*	-	+
				Trachytidae	*Trachytes*	-	+
Trombidiformes	Prostigmata	Eupodides		Eupodidae		+	+
				Tydeidae		+	+
				Ereynetidae		-	+
				Rhagidiidae		+	+
		Anystides	Parsitengonina	Erythraeoidea		-	+
				Trombidellidae		-	+
			Anystina	Anystidae		-	+
		Eleutherengonides	Heterostigmatina	Pygmephoridae		+	+
				Tarsonemidae		-	+
				Microdipidae		-	+
				Scutacaridae		-	+
Collembola	Symphypleona			Arrhopalitidae	*Arrhopalites*	+	-
				Neelidae	*Neelus*	+	-
				Sminthuridae	*Dicyrtoma*	-	+

Arthropleona	Entomobryidae	*Tomocerus*	+	-
		Lepidocyrtus	+	-
		Willowsia	+	-
	Isotomidae	Immature	-	+
		Isotoma	+	+
		Agrenia	+	-
		Folsomia	+	+
		Proisotoma	-	+
		Isotomiella	-	+
	Onychiuridae	*Onychiurus*	+	+
		Tullbergia	+	+
	Hypogastruridae	*Friesea*	-	+
		Pseudachorutes	-	+
Eutardigrada		Unidentified	-	+
	Macrobiotidae	*Macrobiotus*	+	+
	Itaquasconidae	*Diphascon*	-	+
	Hypsibiidae	*Ramazottius*	-	+

Appendix 3: Greenhouse data from the 2004 experiment. Plant ID indicates the plant species and treatment for that pot. P or F for Primrose or Fireweed; C or M for Control soil versus Meanook soil; C or T for Control (root herbivory only) or Treatment (both root and leaf herbivory); and finally the numbers for the replicates. The plant measures were taken at Day 0, before the initial soil core and leaf herbivory was performed. The dry weight of the clipped leaves is also presented.

Plant ID	Species	Treatment (soil/leaves)	Height (cm)	Average branch/ leaf length (cm)	# branches (Fireweed only)	# leaves	# clipped	Dry weight of clipped (g)
PCC1	*Oenothera biennis*	Pasteurized, no clip	29.5	18	n/a	25	-	-
PCC2	*Oenothera biennis*	Pasteurized, no clip	27	16	n/a	25	-	-
PCC3	*Oenothera biennis*	Pasteurized, no clip	25	13	n/a	27	-	-
PCC4	*Oenothera biennis*	Pasteurized, no clip	24.5	15	n/a	26	-	-
PCT1	*Oenothera biennis*	Pasteurized, clipped	23.5	14	n/a	27	16	4.005
PCT2	*Oenothera biennis*	Pasteurized, clipped	19.5	13	n/a	27	16	3.187
PCT3	*Oenothera biennis*	Pasteurized, clipped	19	11	n/a	26	16	3.329
PCT4	*Oenothera biennis*	Pasteurized, clipped	24	14	n/a	29	17	4.388
PMC1	*Oenothera biennis*	Meanook, no clip	23.5	10	n/a	26	-	-
PMC2	*Oenothera biennis*	Meanook, no clip	21.5	11	n/a	27	-	-
PMC3	*Oenothera biennis*	Meanook, no clip	24	12	n/a	27	-	-
PMC4	*Oenothera biennis*	Meanook, no clip	19.5	8	n/a	25	-	-
PMT1	*Oenothera biennis*	Meanook, clipped	24.5	11	n/a	26	16	4.648
PMT2	*Oenothera biennis*	Meanook, clipped	23	10	n/a	28	17	3.709
PMT3	*Oenothera biennis*	Meanook, clipped	23.5	8	n/a	26	16	4.036
PMT4	*Oenothera biennis*	Meanook, clipped	22	10	n/a	25	15	4.068
PMT5	*Oenothera biennis*	Meanook, clipped	24	13	n/a	26	16	4.312
FCC1	*Epilobium angustifolium*	Pasteurized, no clip	54	16	12	201	-	-
FCC2	*Epilobium angustifolium*	Pasteurized, no clip	58	13	8	139	-	-
FCC3	*Epilobium angustifolium*	Pasteurized, no clip	55	21	10	193	-	-

FCC4	*Epilobium angustifolium*	Pasteurized, no clip	63	19	8	111	-	-
FCT1	*Epilobium angustifolium*	Pasteurized, clipped	61	16	13	110	66	0.988
FCT2	*Epilobium angustifolium*	Pasteurized, clipped	60	16	7	88	53	1.448
FCT3	*Epilobium angustifolium*	Pasteurized, clipped	52	20	6	71	43	1.004
FCT4	*Epilobium angustifolium*	Pasteurized, clipped	76	16	7	93	56	2.223
FMC1	*Epilobium angustifolium*	Meanook, no clip	39	15	9	99	-	-
FMC2	*Epilobium angustifolium*	Meanook, no clip	45	20	13	129	-	-
FMC3	*Epilobium angustifolium*	Meanook, no clip	51	19	13	134	-	-
FMC4	*Epilobium angustifolium*	Meanook, no clip	56	21	17	201	-	-
FMT1	*Epilobium angustifolium*	Meanook, clipped	56	11	10	91	55	1.428
FMT2	*Epilobium angustifolium*	Meanook, clipped	46	22	10	107	64	1.23
FMT3	*Epilobium angustifolium*	Meanook, clipped	41	17	19	200	120	1.442
FMT4	*Epilobium angustifolium*	Meanook, clipped	60	13	13	124	74	1.908
FMT5	*Epilobium angustifolium*	Meanook, clipped	50	23	10	116	70	1.625

Appendix 4: Greenhouse data from the 2005 soluble carbon experiment. Plant ID indicates the plant species and treatment for that pot. P or F for Primrose or Fireweed; R, L or C for Root (root herbivory), Leaf (only leaf herbivory), and Control (no herbivory, only cored at the end); and finally the numbers for the replicates. The plant measures were taken at Day 0, before the initial soil core (root herbivory) was performed. Leaf herbivory was done 24 hours after the root herbivory. The dry weight of the clipped leaves is also presented.

Plant ID	Species	Treatment (root/leaves)	Height (cm)	Average branch/leaf length (cm)	# branches (FW)	Avarage # leaves per branch (FW)	# leaves	# clipped	Dry weight of clipped (g)
FR1	*Epilobium angustifolium*	Root	55	20	9	8	121		
FR2	*Epilobium angustifolium*	Root	52	8	14	4	93		
FR3	*Epilobium angustifolium*	Root	42	15	22	7	193		
FR4	*Epilobium angustifolium*	Root	50	17	16	6	121		
FR5	*Epilobium angustifolium*	Root	55	12	14	5	104		
FL1	*Epilobium angustifolium*	Leaf	47	15	19	5	128	77	1.534
FL2	*Epilobium angustifolium*	Leaf	41	15	21	5	127	76	1.799
FL3	*Epilobium angustifolium*	Leaf	66	25	9	9	118	65	2.907
FL4	*Epilobium angustifolium*	Leaf	68	8	8	3	63	38	1.69
FL5	*Epilobium angustifolium*	Leaf	57	22	16	6	129	65	2.638
FC1	*Epilobium angustifolium*	Control	71	27	15	7	135		
FC2	*Epilobium angustifolium*	Control	63	10	14	4	95		
FC3	*Epilobium angustifolium*	Control	63	28	12	10	147		
FC4	*Epilobium angustifolium*	Control	52	12	23	6	170		
FC5	*Epilobium angustifolium*	Control	45	20	14	6	109		
PR1	*Oenothera biennis*	Root	20	12			24		
PR2	*Oenothera biennis*	Root	22	12			26		
PR3	*Oenothera biennis*	Root	22	13			26		
PR4	*Oenothera biennis*	Root	27	16			32		
PR5	*Oenothera biennis*	Root	25	13			33		

PR6	*Oenothera biennis*	Root	23	12	27		
PL1	*Oenothera biennis*	Leaf	21	11	26	16	3.498
PL2	*Oenothera biennis*	Leaf	22	12	25	15	3.702
PL3	*Oenothera biennis*	Leaf	23	13	29	17	4.675
PL4	*Oenothera biennis*	Leaf	23	14	30	18	6.591
PL5	*Oenothera biennis*	Leaf	23	12	25	15	3.558
PL6	*Oenothera biennis*	Leaf	23	14	33	20	3.975
PC1	*Oenothera biennis*	Control	22	14	31		
PC2	*Oenothera biennis*	Control	22	10	28		
PC3	*Oenothera biennis*	Control	26	14	31		
PC4	*Oenothera biennis*	Control	20	10	23		
PC5	*Oenothera biennis*	Control	25	13	26		
PC6	*Oenothera biennis*	Control	22	10	22		

Appendix 5: Greenhouse data for the 2005 experiment on the effect of leaf age at clipping on the soil microbial assemblage. The sample indicates which treatment was done: C, B, O, or Y for Control (no leaves clipped), Both (old and young leaves were clipped), Old (only old leaves were clipped) and Young (only young leaves were clipped). The plant measures were done at Day 0, before the leaf clipping was done.

Sample	Species	Treatment	Height (cm)	# branches	Average # leaves / branch	# leaves	Total # leaves	# clipped	Notes
B1	*Epilobium angustifolium*	Both	38	0	0	28	28	17	
B2	*Epilobium angustifolium*	Both	33	0	0	38	38	23	
B3	*Epilobium angustifolium*	Both	26	2	7	28	42	25	
B4	*Epilobium angustifolium*	Both	49	1	4	33	37	22	
B5	*Epilobium angustifolium*	Both	37	13	8	32	136	82	
C1	*Epilobium angustifolium*	Control	39	2	3	29	35	-	
C2	*Epilobium angustifolium*	Control	46	0	0	34	34	-	
C3	*Epilobium angustifolium*	Control	20	6	4	17	41	-	
C4	*Epilobium angustifolium*	Control	38	2	7	43	57	-	
C5	*Epilobium angustifolium*	Control	32	12	7	31	115	-	
Y1	*Epilobium angustifolium*	Young	28	7	10	9	79	47	4cm stem height (stunted)
Y2	*Epilobium angustifolium*	Young	42	2	6	20	32	19	
Y3	*Epilobium angustifolium*	Young	27	1	2	35	37	22	
Y4	*Epilobium angustifolium*	Young	27	0	0	30	30	18	
Y5	*Epilobium angustifolium*	Young	35	9	9	26	107	64	
O1	*Epilobium angustifolium*	Old	30	2	5	34	44	26	
O2	*Epilobium angustifolium*	Old	29	1	4	39	43	26	
O3	*Epilobium angustifolium*	Old	32	4	14	8	64	38	4cm stem height (stunted)
O4	*Epilobium angustifolium*	Old	38	4	8	19	51	31	
O5	*Epilobium angustifolium*	Old	54	0	0	43	43	26	